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SULFONES OF 7-SILYL- AND 7-GERMYLCEPHALOSPORANATES

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7-Silyl- and 7-germylcephalosporanates in the form of a mixture of 7a and 7b stereoisomers were prepared by the interaction of hydrosilanes and a hydrogermane* with sulfones of tert-butyl esters of 7-diazocephalosporanic acid and 7-diazodesacetoxycephalosporanic acid in the presence of rhodium diacetate. Some of the synthesized substances manifest cytotoxic effects in relation to tumor cells *in vitro*, and also inhibit the catalytic activity of the enzyme elastase.

Structural modification of the side chain of cephalosporins is used extensively to obtain new structural analogs of these antibiotics with improved pharmacological properties. To this end, we undertook an investigation of the introduction of triorganylsilyl and triorganylgermyl groups into position 7 of the sulfones of *tert*-butyl esters of cephalosporin and desacetoxycephalosporin, with the synthesized products to be used in a study of the influence of Group IVA elements on the biological properties of the synthesized substances.

The planned transformation was accomplished on the basis of methodology for the introduction of rhodium-containing carbenoids into the Si–H bond [1-3]. The application of this technique to the synthesis of the target compounds I*a-g* included the diazotization of the sulfones of *tert*-butyl esters of 7-aminocephalosporanic acids (II*a,b*) by means of isopropylnitrile, followed by replacement of the diazo group in III*a,b,* by hydroxyls to obtain IV*a-c* or to obtain the hydrogermane IV*d*, in the presence of $Rh_2(OAc)_4$.

The tendency of the cephalosporin rhodium–carbenoid intermediate V to form by-products in the absence of hydrosilanes (or hydrogermane) determined the order of mixing the reactants. Maximum yields were obtained when the 7-diazocephalosporanates III were added to a dichloromethane solution of the hydrosilane (or hydrogermane) IV and the catalyst at 20°C. After completing the reaction, the sulfones of the 7-silyl- and 7-germylcephalosporanates I*a-g* were separated from the reaction mixture by means of column chromatography, in the form of a mixture of 7α and 7β stereoisomers (Table 1). The ratio of isomers was established by means of HPLC, and their identity by the PMR spectra, which contained characteristic signals of the protons C_6–H and C_7–H with spin–spin coupling constants J = 5 Hz for the *cis* or 7-β stereoisomer and J = 2 Hz for the *trans* or 7α stereoisomer (Table 2).

It is known that in the process of replacing the diazo group, the nonplanarity of the condensed cephem ring of the cephalosporin favors preferential formation of the 7α isomers as a result of stereoselectivity in the approach of the carbanions from the α-side of the β-lactam ring. However, analysis of the ratios of 7α and 7β isomers indicates that this is not a significant factor; also, there is no significant influence of substituent size or the nature of the M–H bond in compounds IV*a-d* on the stereoselectivity of this reaction (see Table 1).

When the 7-silylcephalosporanates I*b* and I*f* are treated briefly with trifluoroacetic acid, the ester protective group is split off. From the reaction medium, by means of preparative column chromatography, we isolated the corresponding cephalosporanic acids VI, characterized as individual 7α stereoisomers by their PMR spectra (see Table 2).

The cytotoxic properties of the synthesized compounds were tested on two standard lines of tumor cells: HT-1080 (human fibrosarcoma) and MG-22A (mouse hepatoma). We also investigated the influence of these substances on the

*Generic terms for compounds with the general formula $HSiX_3$ or $HGeX_3$—Translator.

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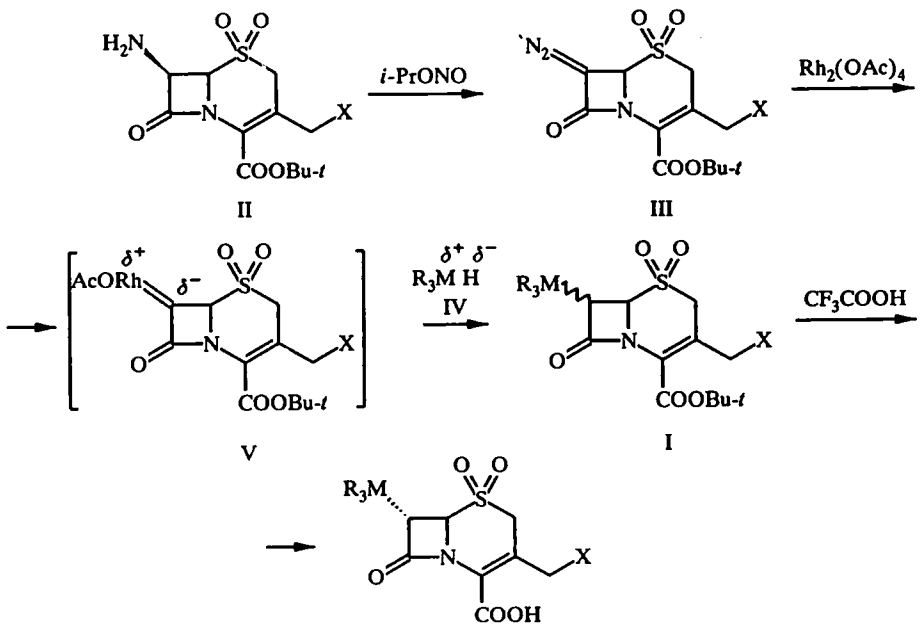

I a X = H, R_3M = Et_3Si; b X = OAc, R_3M = Et_3Si; c X = H, R_3M = t-$BuMe_2Si$; d X = OAc, R_3M = t-$BuMe_2Si$; e X = H, R_3M = $PhMe_2Si$; f X = OAc, R_3M = $PhMe_2Si$; g X = OAc, R_3M = Et_3Ge.
II, III, V a X = H, b X = OAc. IV a R_3M = Et_3Si, b R_3M = t-$BuMe_2Si$, c R_3M = $PhMe_2Si$, d R_3M = Et_3Ge.
VI a X = OAc, R_3M = Et_3Si; b X = OAc, R_3M = $PhMe_2Si$

amidolytic activity of Porcine Pancreas Elastase (Type III) in relation to the *p*-nitroanilide of the standard tetrapeptide N-methoxysuccinyl-ala-ala-pro-val as the substrate. The results of these studies are presented in Table 3.

From an analysis of the relation between structure and activity for these substances, we can draw the following conclusions: Cephalosporins containing an acetoxy group manifest higher activity as cytotoxic substances and inhibitors of elastase in comparison with the corresponding desacetoxycephalosporins; triethylsilyl and triethylgermyl groups are effective in suppressing the growth of tumor cells *in vitro* at lower concentrations than are required with other 7-substituted cephalosporanates.

EXPERIMENTAL

PMR spectra were obtained in a Bruker WH-90/DS spectrometer (90 MHz) in $CDCl_3$, internal standard TMS; the IR spectra were obtained in a Perkin-Elmer 580B spectrometer, in white mineral oil. Elemental analyses were performed in a Carlo Erba 1108 analyzer. The HPLC data were obtained in a Du Pont Model 8800 instrument equipped with a UV detector (λ = 254 nm) and a column (4.6×250 mm) packed with Supelcosil LC-Si phase (Symmetry C_{18}), in a system of hexane–ethyl acetate 4:1, throughput rate 1.5-2.0 ml/min. The course of the reaction was monitored by TLC on Merck Kieselgel plates with UV development. The preparative column chromatography employed Merck Kieselgel silica gel (0.063-0.230 mm). In these experiments, we used reagents and materials from Aldrich, Acros, and Sigma.

The ratio of stereoisomers, the empirical formulas, elemental analyses, and values of $\upsilon_{C=O}$ of the β-lactam carbonyl in the IR spectrum are listed in Table 1.

The sulfone of the *tert*-butyl ester of 7-diazodesacetoxycephalosporanic acid (III*a*) and the sulfone of the *tert*-butyl ester of 7-diazocephalosporanic acid (III*b*) were synthesized by means of a procedure described in [4].

Sulfone of *tert*-Butyl Ester of 7-Triethylsilyldesacetoxycephalosporanic Acid (I*a*). To a solution of triethylsilane (453 μl, 2.8 mmoles) in 5 ml of dry CH_2Cl_2, a catalytic quantity of $Rh_2(OAc)_4$ was added; then, over the course of 1 h, there was added the sulfone of the *tert*-butyl ester of 7-diazodesacetoxycephalosporanic acid (300 mg, 0.95 mmole) dissolved in 2 ml of dry CH_2Cl_2. The mixture was stirred 3 h at room temperature, after which the solvent

TABLE 1. Characteristics of 7-Silyl- and 7-Germyl-Substituted Cephalosporanates

Compound	R_3M	X	Ratio 7α- and 7β-isomers*	Empirical formula	Found, % / Calculated, %			mp, °C	IR spectra, cm^{-1}, $\nu_{C=O}$ (β-lactam)	R_f[3]
					C	H	N			
Ia	Et_3Si	H	79 : 21	$C_{18}H_{31}NO_5SSi$	53,47 / 53,83	7,62 / 7,78	3,53 / 3,49	96...97	1800	0,60
Ib	Et_3Si	OAc	65 : 35	$C_{20}H_{33}NO_7SSi$	52,09 / 52,27	7,24 / 7,23	3,31 / 3,05	58...60	1780	0,71
Ic	t-$BuMe_2Si$	H	70 : 30	$C_{18}H_{31}NO_5SSi \cdot 0{,}9C_6H_{14}$	58,68 / 58,66	8,87 / 9,16	2,98 / 2,92	148...151	1770	0,48
Id	t-$BuMe_2Si$	OAc	75 : 25	$C_{20}H_{33}NO_7SSi \cdot 0{,}1C_6H_{14}$	52,94 / 52,84	7,44 / 7,35	3,04 / 2,99	95...98	1790	0,34
Ie	$PhMe_2Si$	H	70 : 30	$C_{20}H_{27}NO_5SSi$	56,70 / 56,98	6,61 / 6,45	3,20 / 3,32	55...56	1780	0,57
If	$PhMe_2Si$	OAc	77 : 23	$C_{22}H_{29}NO_7SSi$	54,83 / 55,10	6,20 / 6,09	3,08 / 2,92	100...103	1770	0,57
Ig	Et_3Ge	OAc	62 : 38	$C_{20}H_{33}NO_7SGe \cdot 0{,}25C_6H_{14}$	48,52 / 48,86	6,68 / 6,99	2,99 / 2,66	Oil	1780	0,43
VIa	Et_3Si	OAc	100 : 0	$C_{16}H_{25}NO_7SSi$	*2			85...87	1780	0,50*4
VIb	$PhMe_2Si$	OAc	100 : 0	$C_{18}H_{21}NO_7SSi$	*2			45...47	1780	0,50*4

*Data from HPLC analysis.
*2According to HPLC data, the content of principal substance is 93%.
*3TLC, eluent hexane–ethyl acetate, 2:1.
*4TLC, eluent hexane–ethyl acetate, 1:1.

TABLE 2. PMR Spectra of Synthesized Compounds

Compound (stereoisomer)	Chemical shift (δ) and spin-spin coupling constant (J) protons (ppm, Hz)							
	C_6-H	C_7-H	SO_2CH_2	3-CH_3	3-$OCOCH_3$	$COOC(CH_3)_3$	COOH	R_3M
Ia (α)	4,53, br.s	3,48, d, J = 2	3,60, 3,86 AB-q, J = 19	2,02				0,57...1,22 (15H, m, $3C_2H_5$)
Ia (β)	4,80, d, J = 4	3,62, d, J = 4	3,40...3,7	1,94				0,57...1,22 (15H, m, $3C_2H_5$)
Ib (α)	4,62, br.s	3,60, s	3,62, 4,02 AB-q, J = 20		2,11	1,62		0,64...1,17 (15H, m, $3C_2H_5$)
Ib (β)	4,82, d, J = 4	3,57, d, J = 4	3,75, 4,00 AB-q, J = 18		2,11	1,62		0,64...1,17 (15H, m, $3C_2H_5$)
Ic (α)	4,48, d, J = 1	3,35, d, J = 1	3,51, 3,86 AB-q, J = 18	1,97		1,46		0,15 (6H, d, J = 4 Hz, $2CH_3$); 0,95 (9H, s, $(CH_3)_3$)
Ic (β)	4,77, d, J = 4	3,42, d, J = 4	3,42, 3,62 AB-q, J = 15	1,86		1,48		0,22 (6H, d, J = 1 Hz, $2CH_3$); 0,91 (9H, s, $(CH_3)_3$)
Id (α)	4,48, d, J = 1	3,51, d, J = 1	3,68, 3,97 AB-q, J = 18		2,11	1,55		0,22 (6H, d, J = 3 Hz, $2CH_3$); 1,02 (9H, s, $(CH_3)_3$)
Id (β)	4,51...5,00, m	3,57...3,93, m	3,57...3,93, m		2,11	1,60		0,33 (6H, d, J = 2 Hz, $2CH_3$); 0,98 (9H, s, $(CH_3)_3$)
Ie (α)	4,40, br.s	3,64, s	3,35, 3,71 AB-q, J = 19	2,00				0,51 (6H, s, $2CH_3$); 7,30...7,71 (5H, m, C_6H_5)
Ie (β)	4,77, d, J = 5	3,55, d, J = 5	3,30...3,80, m	2,00		1,53		0,62 (6H, d, J = 3 Hz, $2CH_3$); 7,30...7,71 (5H, m, C_6H_5)
If (α)	4,35, br.s	3,60, br.s	3,44, 3,88 AB-q, J = 19		2,04	1,53		0,60 (6H, d, J = 3 Hz, $2CH_3$); 7,26...7,66 (5H, m, C_6H_5)
If (β)	4,80, d, J = 5	3,68, d, J = 5	3,55, 3,78 AB-q, J = 14		2,04	1,53		0,60 (6H, s, $2CH_3$); 7,26...7,66 (5H, m, C_6H_5)
Ig (α)	4,55, br.s.	3,55, br.s	3,71, 3,95 AB-q, J = 18		2,08	1,53		0,53...1,15 (15H, m, $3C_2H_5$)
Ig (β)	4,50...5,20, m	3,50...4,00	3,50...4,00 m		2,08	1,53		0,53...1,15 (15H, m, $3C_2H_5$)
VIa (α)	4,66, d, J = 1	3,44, d, J = 1	3,79, 4,00 AB-q, J = 18		2,06		7,26	0,53...1,15 (15H, m, $3C_2H_5$)
VIb (α)	4,53, d, J = 1	3,64, d, J = 1	3,77, 3,91 AB-q, J = 19		2,04		7,77	0,51 (6H, s, $2CH_3$); 7,28...7,77 (5H, m, C_6H_5)

TABLE 3. Biological Properties of 7-Silyl- and 7-Germylcephalosporanates

Compound	Inhibition of elastase, IC_{50} (mmoles)*	Cytotoxic effect in relation to indicated tumor cells (μg/ml)			
		MG-22A		HT-1080	
		TD_{50} (CV)*2	TD_{50} (MTT)*3	TD_{50} (CV)	TD_{50} (MTT)
Ia	—	>100	>100	100	>100
Ib	—	53	48	69	67
Ic	—	>100	>100	62	96
Id	—	>100	>100	74	86
Ie	1,00	>100	>100	>100	>100
If	0,33	71	65	>100	>100
Ig	—	49	42	52	49
VIb	0,33	71	65	>100	>100

*Concentration (in mmoles) giving 50% inhibition of amidolytic activity of Porcine Pancreas Elastase (Type III), with the use of the *p*-nitroanilide of N-methoxysuccinyl-ala-ala-pro-val as the substrate.

*2Lethal concentration (in μg/ml) for 50% of the cells; staining with CV, crystal violet.

*3Lethal concentration (in μg/ml) for 50% of the cells; staining with MTT, 3-(4,5-dimethylthiazolyl-2)-2,5-diphenyltetrazolium bromide.

was evaporated under reduced pressure. The residue was chromatographed in a column with silica gel (eluent hexane–ethyl acetate, 2:1). The fractions with *Rf* 0.60 were combined and evaporated down. Treatment of the oily residue with petroleum ether gave 100 mg of the crystalline substance. Yield 26%.

Sulfone of *tert*-Butyl Ester of 7-Triethylsilylcephalosporanic Acid (*Ib*). To a solution of triethylsilane (776 μl, 4.86 mmoles) in 5 ml of CH_2Cl_2, a catalytic quantity of $Rh_2(OAc)_4$ was added; then, over the course of 1 h, there was added the sulfone of the *tert*-butyl ester of 7-diazocephalosporanic acid (600 mg, 1.62 mmoles) dissolved in 2 ml of dry CH_2Cl_2. The mixture was stirred 3 h at room temperature, after which the solvent was evaporated under reduced pressure. The residue was chromatographed in a column with silica gel (eluent hexane–ethyl acetate, 2:1). The fractions with *Rf* 0.71 were combined and evaporated down. Treatment of the oily residue with petroleum ether yielded 210 mg of the crystalline substance. Yield 28%.

Sulfone of *tert*-butyl ester of 7-*tert*-butyldimethylsilyldesacetoxycephalosporanic acid (Ic) was obtained in the same manner as compound I*a*, from *tert*-butyldimethylsilane and the sulfone of the *tert*-butyl ester of 7-diazodesacetoxycephalosporanic acid. Yield 12%.

Sulfone of *tert*-butyl ester of 7-*tert*-butyldimethylsilylcephalosporanic acid (Id) was obtained in the same manner as compound I*b*, from *tert*-butyldimethylsilane and the sulfone of the *tert*-butyl ester of 7-diazocephalosporanic acid. Yield 23%.

Sulfone of *tert*-butyl ester of 7-phenyldimethylsilyldesacetoxycephalosporanic acid (Ie) was obtained in the same manner as compound I*a*, from phenyldimethylsilane and the sulfone of the *tert*-butyl ester of 7-diazodesacetoxycephalosporanic acid. Yield 19%.

Sulfone of *tert*-butyl ester of 7-phenyldimethylsilylcephalosporanic acid (If) was obtained in the same manner as compound I*b*, from phenyldimethylsilane and the sulfone of the *tert*-butyl ester of 7-diazocephalosporanic acid. Yield 33%.

Sulfone of *tert*-butyl ester of 7-triethylgermylcephalosporanic acid (Ig) was obtained in the same manner as compound I*b*, from triethylgermane and the sulfone of the *tert*-butyl ester of 7-diazocephalosporanic acid. Yield 15%.

Sulfone of 7-triethylsilylcephalosporanic acid (VI*a*). A solution of the sulfone of the *tert*-butyl ester of 7-triethylsilylcephalosporanic acid I*b* (300 mg, 0.65 mmole) in 3 ml of trifluoroacetic acid was held for 1 h at room temperature, after which the solvent was evaporated under reduced pressure. The residue was chromatographed in a column with

silica gel (eluent hexane–ethyl acetate, 1:1). The fractions with R_f 0.50 were combined and evaporated down, obtaining 210 mg of a crystalline substance. Yield 35%.

Sulfone of 7-phenyldimethylsilylcephalosporanic acid (VI*b*) was obtained in the same manner as compound VI*a*, from the sulfone of the *tert*-butyl ester of 7-phenyldimethylsilylcephalosporanic acid I*f*. Yield 50%.

Biological Tests. The influence of compounds I*a* and I*b* on the catalytic properties of Porcine Pancreas Elastase (Type III) with respect to the substrate *p*-nitroanilide of N-methoxysuccinyl-ala-ala-pro-val was determined by means of a standard procedure using a Tetertek Multiscan MCC/340 horizontal spectrophotometer to measure the optical density (method described in [5]).

The cytotoxic properties of the compounds were investigated on cultures of monolayer cells grown in 96 cutout panels in standard medium without indicator or antibiotics, following a standard procedure given in [6]. The numbers of live cells were determined by two colorimetric methods on the basis of the intensity of staining of the cell membranes by crystal violet and staining of the cell medium by 3-(4,5-dimethylthiazolyl-2)-2,5-diphenyltetrazolium bromide, characterizing the intensity of redox processes of the mitochondrial enzymes of the cells, in comparison with the control.

The control cells (without test substances) were grown on a separate panel.

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