gate this matter further several sets of isomers of RSO_2SR' and $R'SO_2SR$ were prepared, as well as a number of other mixed and symmetrical thiolsulfonates to relate structure with antimicrobial activity.

The unsubstituted S-alkyl members were prepared by alkylation.[7]

$$RSO_2SK + R'Br \longrightarrow RSO_2SR' + KBr$$

The S-tosyl and S-trichlorovinyl esters were prepared by reacting the sulfenyl chloride with the sulfinate[8]

$$RSO_2M + C_7H_7SCl \longrightarrow RSO_2SC_7H_7 + MCl$$

where M is silver or zinc.

The bactericidal activity of these thiolsulfonates is given in Table I. A number of the compounds, namely those of lower molecular weight and the acetylenic derivatives, have high activity. Table II compares the

TABLE II

BACTERIOSTATIC ACTIVITY OF THIOLSULFONATES

RSO_2SR'

R	R'	MID[a]
	Symmetrical	
CH_3	CH_3	12
C_8H_{17}	C_8H_{17}	40
$C_{16}H_{33}$	$C_{16}H_{33}$	200
C_7H_7	C_7H_7	500
	Unsymmetrical	
CH_3	C_8H_{17}	6
C_8H_{17}	CH_3	12
CH_3	$C_{16}H_{33}$	1000
$C_{16}H_{33}$	CH_3	>1000
CH_3	C_7H_7	40
C_7H_7	CH_3	34
C_8H_{17}	$C_{16}H_{33}$	250
$C_{16}H_{33}$	C_8H_{17}	1000
C_8H_{17}	C_7H_7	8
C_7H_7	C_8H_{17}	0.3
$C_{16}H_{33}$	C_7H_7	200
C_7H_7	$C_{16}H_{33}$	200

[a] Minimum inhibitory dose in parts per million to *Staphylococcus aureus*.

bacteriostatic action of a series of symmetrical thiolsulfonates and mixed isomers. It can be concluded from these data that the whole molecule enters into the toxic mechanism and that, although the toxicity toward bacteria of some of the isomers differs, neither the RSO_2- nor the RS- alone can be considered to be the active moiety. Table III presents data on a series of trichlorovinyl thiolsulfonates which were found to have very high bactericidal activity.

TABLE III

BACTERICIDAL ACTIVITY OF TRICHLOROVINYL THIOLSULFONATES

$RSO_2SCCl{=}CCl_2$

R	MLD[a]
CH_3	2
C_2H_5	8
n-C_3H_7	8
n-C_4H_9	8
n-C_8H_{17}	250
C_6H_5	40

[a] Minimum lethal dose in parts per million to *Staphylococcus aureus*.

(7) R. Otto, *Ber.*, **13**, 1282 (1880).

(8) B. G. Boldyrev, *Dokl. Akad. Nauk SSSR.* **131**, 1331 (1960).

Experimental[9]

A.—The preparation of *n*-butyl *p*-toluenethiolsulfonate illustrates the general synthesis of the S-alkyl thiolsulfonates. To a 200-ml. two-necked flask was added a mixture of 12.2 g. (0.05 mole) of recrystallized potassium *p*-toluenethiolsulfonate,[10] 97 ml. of acetone, and 3 ml. of water. The salt was crushed in the solvents to insure small particle size. The flask was fitted with a stirrer, condenser, and heating mantle. To the flask was added at once 6.85 g. of *n*-butyl bromide and the mixture stirred and heated for 20 hr. After completion of the reaction the mixture was diluted with water and transferred to a 300-ml. separatory funnel. The heavy organic layer was removed, diluted with ether, and dried over sodium sulfate. Filtration of the ether solution and evaporation of the ether gave 10.5 g. of crude product. The compound was purified by thin layer chromatography by coating 20-cm. square glass plates to a thickness of 250 μ with silica gel. After activation of the plates by heating for 30 min. at 110°, the crude compound was applied by a small pipet about 2 cm. from the bottom edge, and the plate developed in a solvent mixture of benzene–petroleum ether–chloroform (8:8:1 by volume). After the solvent had risen to the top, the plates were removed, and one was sprayed with 10% sulfuric acid and charred at 110°. The position of the compound on the charred plate indicated where to remove the silica from the other plates. Ether extraction of the silica from 10–20 such plates and evaporation of the ether provided a sufficient amount of compound for analysis and microbiological testing.

B.—The preparation of *p*-tosyl *n*-octanethiolsulfonate illustrates the synthesis of the S-tosyl thiolsulfonates. A 250-ml. erlenmeyer flask containing 50 ml. of absolute ether was set on a magnetic stirrer in the dark and 12.55 g. (0.044 mole, approx. 10% excess) of silver octanesulfinate[11] added. To the stirring suspension 6.85 g. (0.040 mole) of *p*-toluenesulfenyl chloride,[12] dissolved in 45 ml. of absolute ether, was added in small amounts over a period of 2–3 min. During the addition the temperature rose, causing the ether to boil and the orange-red solution of sulfenyl chloride to lose some of its color. After standing 15 min. in the dark the reaction mixture was filtered giving 9.5 g. of yellow solid; the theoretical requirement of silver chloride is 6.87 g. The pale yellow filtrate upon evaporation of the ether gave 8.8 g. of light yellow oil which was purified, as above, by thin layer chromatography.

The S-trichlorovinyl esters were made by reaction of the zinc sulfinate with 1,2,2,2-tetrachloroethanesulfenyl chloride which has been described elsewhere.[13]

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Antiviral Compounds. IX. Steroid Derivatives

G. CAVALLINI,[1a] E. MASSARANI,[1b] AND D. NARDI

Medicinal Chemistry Research Laboratories, Vister-Casatenovo Brianza (Como), Italy

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In previous articles we had recorded the observation that a combination of a proper functional group with an appropriate radical may lead to compounds which display *in vivo* pharmacodynamic activities.[2]

(1) (a) Deceased. (b) Author to whom inquiries should be addressed, Research Division, Recordati S.p.A., Milan, Italy.

(2) G. Cavallini and E. Massarani, *J. Med. Pharm. Chem.*, **1**, 365 (1959).

TABLE I[a]

	Embryonated eggs[d]			Tissue culture[d]				Mice	
	Virucidal activity	Virustatic activity		Virucidal activity		Virustatic activity			
	A-PR8 virus		MTD[b] γ/ml.	Polio type 1	Vaccin	Polio type 1	Vacciniala	LD_{50} mg./kg.	MHV_3[c] Craig virus
I	0	0	50	0	0	0	0	1000	0
II	1	1	100	0	0	0	0	>1500	89
III	0	0	100	0	0	0	0	>1500	26
IV	1	0	100	0	0	0	0	>1500	58
V	7Δ	0	50	0	2Δ	0	1	>1500	0
VI	7Δ	4Δ	50	0	2Δ	1	0	>1500	312Δ
VII	7Δ	2Δ	100	0	0	0	1	>1500	41
VIII	7Δ	1	100	0	0	0	0	>1500	86
IX	5Δ	1	100	0	0	0	0	>1500	0
X	5Δ	3Δ	100	0	1	1	1	>1500	168Δ
XII	5Δ	0	50	0	0	0	0	1500	37
XIII	5Δ	0	50	0	0	1	1	1500	24
XIV	4Δ	1	25	0	0	0	1	1500	148Δ

[a] The data which are statistically valid are indicated by Δ. [b] Maximal tolerated dose. [c] Difference between averages of the determinations of sorbitol–dehydrogenase activity (according to Gerlach) in the plasma of treated animals and in that of control animals. The significance was calculated by Student's "t." [d] The values are the differences of titers corresponding to logarithmic units.

TABLE II

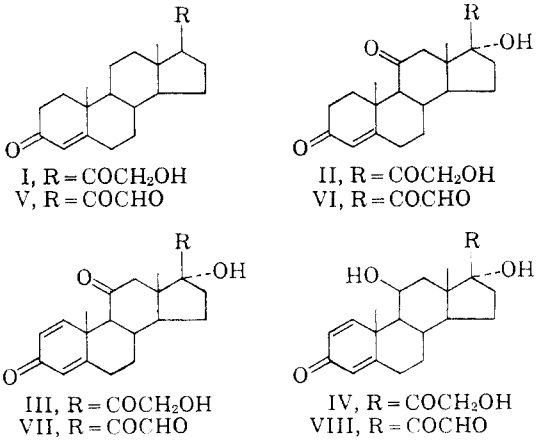
SCHIFF'S BASES AND N,N-DIACETALS OF STEROIDAL GLYOXALS

No.	Compd.	Yield, %	M.p., °C. dec.	Solvent of crystn.[a]	Molecular composition	Calcd., % C	H	N	Found, % C	H	N
IX	Δ4-Pregnen-21-al-3,20-dione N,N-*p*-carboxyanilino diacetal	60	145–148	E	$C_{35}H_{40}N_2O_6 \cdot H_2O$	69.74	7.02	4.65	69.81	7.04	5.02
X	Δ4-Pregnen-17-ol-21-al-3,11,20-trione N,N-*p*-carboxyanilino diacetal	50	155–158	E	$C_{35}H_{38}N_2O_8 \cdot C_2H_5OH \cdot H_2O$[b]	65.45	6.83	4.13	65.70	7.11	4.00
XI	Δ1,4-Pregnadien-17-ol-21-al-3,11,20-trione N,N-*p*-carboxyanilino diacetal	75	145–146	E	$C_{35}H_{36}N_2O_8 \cdot C_2H_5OH \cdot H_2O$[b]	65.66	6.55	4.14	65.63	6.49	4.13
XII	Δ4-Pregnene-3,11-dion-17-ol-17-glyoxilidene cyclohexylamine[c]	92	191–192	E	$C_{27}H_{37}NO_4$	73.77	8.48	3.19	73.51	8.31	3.12
XIII	Δ1,4-Pregnadiene-3,11-dion-17-ol-17-glyoxilidene cyclohexylamine	70	193	E	$C_{27}H_{35}NO_4$	74.11	8.06	3.20	73.80	7.86	3.33
XIV	Δ4-Pregnene-3,11-dion-17-ol-17-glyoxilidene *p*-aminophenol	66	204–205	*d*	$C_{27}H_{31}NO_5$	72.14	6.95	3.12	72.19	6.82	3.02

[a] E = ethanol. [b] *Anal.* Calcd.: C_2H_5O, 6.63. Found: C_2H_5O, 6.67. [c] The reaction was carried out for 2 hr. [d] The compound was obtained by evaporation *in vacuo* from ethanol at 20°; the residue was washed with ethyl ether.

By attaching suitable functional groups to hormonal structures, compounds with a wide variety of such activities were obtained,[3] while the hormonal properties of the "supporting moiety" were abolished. Since we had also found that glyoxals derived from several polycyclic systems are active antiviral agents *in vivo*,[4] we have studied α-ketoaldehydes V–VIII derived from steroid structures and compared them to the corresponding α-ketols (I–IV) whose hormonal activities are well known. In addition, several Schiff's bases and N,N-diacetals of the ketoaldehydes were also prepared and tested since a more potent and specific antiviral activity had been noted in other similarly protected glyoxal derivatives in other series.[4a]

All the compounds were tested in embryonated chicken eggs against influenza virus A-PR8, in tissue cultures against type I poliomyelitis and vaccinia virus, and in mice against hepatitis MHV_3 virus (Craig strain). The methods employed have been described previously.[4b] The results are listed in Table I. None of the ketols (I–IV) exhibited antiviral activity under the conditions of the tests. By contrast, all the glyoxals and their Schiff's bases and N,N-diacetals proved to be very active against influenza A-PR8 virus in chick embryos. Three of the com-



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pounds (VI, VII, and X) were also virustatic. Compounds V and VI were virucidal against vaccinia in tissue culture; VI, X, and XIV showed activity against the hepatitis viral strain in mice. These results support the soundness of the assumption on which this research was planned, that ketoaldehydes derived from steroidal components would be antiviral.

Experimental[5]

All α-ketoaldehydes discussed in this note have been described in the literature. We prepared them in good yield by oxidation of the corresponding ketols with oxygen in aqueous methanol solution. Chemical and physical characteristics (especially infrared frequencies and rotatory indices) were in accord with literature data.

Preparation of Schiff's Bases and N,N-Diacetals.—A mixture of the ketoaldehyde (1 mmole) and the respective primary amine (1–2 mmoles) in 10 ml. of ethanol was stirred at 20° for 24 hr., and the solution was concentrated until crystallization took place.

(5) All melting points are corrected.

The Preparation of Penicilloyl–Polylysines, Skin Test Reagents for the Clinical Evaluation of Penicillin Hypersensitivity

BERNARD B. LEVINE

Department of Medicine, New York University School of Medicine, New York, New York

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The benzylpenicilloyl (BPO) group has been demonstrated to be the major haptenic antigenic determinant of benzylpenicillin (PG) hypersensitivity.[1-3] Multivalent benzylpenicilloyl–polylysine (BPO–PLL) conjugates have been found to be effective elicitors of allergic skin reactions of the wheal-and-flare reactions in a significant percentage of patients with histories of allergy to PG.[2,3] These materials are accordingly promising skin test reagents for the clinical evaluation of penicillin hypersensitivity. Penicilloyl–PLL conjugates have been prepared previously by reaction of penicillenic acids with polylysine.[1-4] This procedure is tedious and results in conjugates contaminated with penicillenic acid groupings and with other impurities. This paper reports a new and simple method for the preparation of comparatively clean succinoylated multivalent penicilloyl–PLL(S) conjugates of different extents of conjugation directly from penicillins. This method is based on the known reaction of penicillins with aliphatic amines at high pH to form penicilloylamines.[5,6] Parker and Thiel have recently published a method of preparation of maximally coupled, unsuccinoylated penicilloyl–PLL conjugates based on this reaction.[7]

Table I shows the relation between mole ratios of reactants and the extents of conjugation of penicilloyl–PLL(S) conjugates which were prepared from PLL preparations of four different degrees of polymerization and from four different penicillins. Over 100 preparations have been made with similar results. Under the same conditions, penicillins were made to react with poly-D-lysine to form succinoylated multivalent penicilloyl–poly-D-lysine conjugates. This preparative method thus appears to be a general one.

The extents of conjugation listed in Table I are 7% too low, as 7% of the penicilloyl groups undergo N^4-thiazolidine acylation during the succinoylation reaction. N^4-Acylated penicilloyl residues do not undergo the penamaldate reaction[5,6] which is the basis of the penicilloyl assay. A maximum of only 60% of the NH_2 groups of PLL could be coupled with penicilloyl groups, probably because of steric interference from the bulky penicilloyl groups. Succinoylation, under the conditions given in the Experimental section, coupled at least 97% of the NH_2 groups, as determined by formol titrations.[8] The conjugate solutions were found to be free from unreacted penicillins by bioassay[9] and free from benzylpenicilloic acid by arsenomolybdate reduction.[10] The ultraviolet absorption spectrum of benzylpenicilloyl–PLL(S) conjugates showed absorption peaks at 278 mμ which corresponds to the presence, in the conjugates, of penamaldoyl groups[5] formed by rearrangement of penicilloyl groups.[5] The optical densities at 280 mμ of some typical conjugate solutions indicate that 1 to 3% of the penicilloyl groups contained in the conjugates exist as the tautomeric penamaldate form. The absorption spectra show also superimposed peaks at λ 258 and 264 mμ corresponding to the benzyl side chain,[5] and another peak at λ 268 mμ which may indicate trace quantities of penaldate groups.[5] There were no detectible benzylpenicillenic acid disulfide chromophoric groupings detectible in the conjugate solutions as evidenced by the absence of absorption maxima in the 310–340 mμ region.[5]

Optical rotations of the conjugate solutions corrected for the contribution of succinoylated PLL yielded $[\alpha]^{25}D$ +0.96° for $1 \times 10^{-2}M$ benzylpenicilloyl contained in a typical benzylpenicilloyl–PLL(S) conjugate, a value in excellent agreement with the molar specific rotations of α-diastereoisomeric crystalline univalent benzylpenicilloylamines.[6] This finding indicates that the penicilloyl groups contained in the conjugates prepared by the method given here are entirely, or predominantly, α-diastereoisomers, the expected diastereoisomeric product of the reaction of penicillins with amines at high pH.[5,6] In contrast, penicilloyl–polylysines prepared from penicillenic acids are diastereoisomeric mixtures.[1] Optical rotations of benzylpenicilloyl–PLL(S) solutions (BPO_{80}–PLL_{102}(S) and BPO_{60}–PLL_{286}(S)) taken at decreasing pH showed

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