INVESTIGATIONS IN THE AREA OF SEMISYNTHETIC PENICILLINS. III. SOME METHOXY- AND DIALKOXYPHENYLBENZYLPENICILLINS

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UDC 615.334

Many of the earlier known penicillins contain di- and tri- substituted acetic acids in the side chain. Triphenylmethylpenicillin [1], which has the same activity in vitro against pathogenic staphylococci regardless of their ability to produce penicillinase, was the first of the latter to display a clearly expressed resistance toward staphylococcus penicillinase. It was inferior in activity to penicillin D with regard to susceptible staphylococci but proved to be more active with regard to resistant strains.

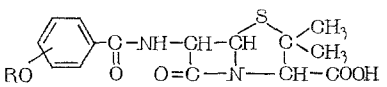
This and analogous facts led us to feel that this resistance to the enzyme was caused by the presence of the three substituents in the α-position. It should be mentioned that the resistance to the enzyme reached a maximum for the triphenylmethyl derivative.

Many of the penicillins of this type displayed a high activity only in vitro toward the staphylococci resistant to benzylpenicillin; they were not active in experiments on animals, which can evidently be explained by their poor absorbability.

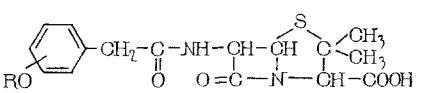
These data changed the direction of our investigations and again led to the synthesis of sterically hindered acids, but just through substituting a hydrogen in the aromatic ring.

2,6-Disubstituted benzoic acids [2] were chosen as the starting products. This choice gave good results and led to the synthesis of 2,6-dimethoxyphenylpenicillin (methicillin), which was highly resistant to penicillinase and active toward staphylococci both in vitro and in vivo [3].

Data on the synthesis and the investigation of the properties of phenyl- and benzylpenicillins (I-IV and V-VII), which have methoxyl groups as the substituents in the ortho, meta, and para positions of the benzene ring (Table 1), are presented in this report:

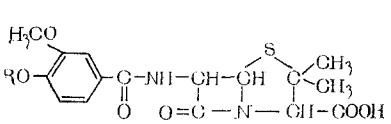


I-IV

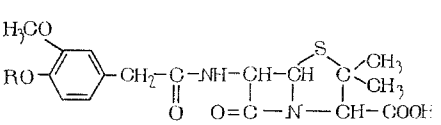


V-VII

Dialkoxyphenyl- and benzylpenicillins with the structure of VIII-XIII and XIV-XIX (Tables 2 and 3) were also prepared:



VIII-XIII



XIV-XIX

Institute of Fine Organic Chemistry, Academy of Sciences of the Armenian SSR. Erevan. Translated from Khimiko-Farmatsevticheskii Zhurnal, No. 3, Vol. 4, pp. 5-10, March, 1970. Original article submitted September 11, 1969.

TABLE 1. Methoxyphenyl- and Benzylpenicillins

Compound	RO	Yield (in %)	Melting point (in deg)*	Found (in %)				Empirical formula	Calculated (in %)				$[\alpha]_D^{20}$ (concn. 0.87, water)	R_f
				C	H	N	S		C	H	N	S		
I	—	75,3	89—90	56,67	5,38	8,45	9,72	$C_{15}H_{16}N_2O_4S$	56,55	5,00	8,74	10,00	185,9	0,59
II	o-CH_3O	72,5	115—6	55,18	5,50	8,02	9,25	$C_{16}H_{18}N_2O_5S$	54,84	5,17	7,99	9,15	200,2	0,62
III	m-CH_3O	66,4	93—4	55,09	5,15	7,90	8,87	$C_{16}H_{18}N_2O_5S$	54,84	5,17	7,99	9,15	159,4	0,70
IV	p-CH_3O	68,8	80—1	54,90	5,00	8,21	8,97	$C_{16}H_{18}N_2O_5S$	54,84	5,17	7,99	9,15	220,3	0,58
V	o-CH_3O	59,3	121—2	56,18	5,39	7,50	8,99	$C_{17}H_{20}N_2O_5S$	56,02	5,53	7,68	8,79	159,4	0,85
VI	m-CH_3O	63,4	92—3	55,84	5,65	7,70	8,44	$C_{17}H_{20}N_2O_5S$	56,02	5,53	7,68	8,79	239,0	0,81
VII	p-CH_3O	62,5	77—8	55,90	5,81	7,39	8,34	$C_{17}H_{20}N_2O_5S$	56,02	5,53	7,68	8,79	157,4	0,60

*Melt with decomposition.

TABLE 2. m-Methoxy-p-alkoxyphenylpenicillin

Compound	R	Yield (in %)	Melting point (in deg)*	Found (in %)				Empirical formula	Calculated (in %)				$[\alpha]_D^{20}$ (concn. 0.87, water)	R_f
				C	H	N	S		C	H	N	S		
VIII	CH_3	70,4	150—1	53,74	5,04	7,03	8,21	$C_{17}H_{20}N_2O_6S$	53,67	5,29	7,36	8,42	212,5	0,62
IX	C_2H_5	69,5	142—3	54,69	5,53	7,02	8,14	$C_{18}H_{22}N_2O_6S$	54,80	5,62	7,15	8,12	159,4	0,72
X	n-C_3H_7	75,3	138—9	55,69	5,58	6,82	8,06	$C_{19}H_{24}N_2O_6S$	55,86	5,92	6,85	7,85	212,5	0,71
XI	iso-C_3H_7	71,2	108—9	56,08	6,99	6,81	8,09	$C_{19}H_{24}N_2O_6S$	55,86	5,92	6,85	7,85	289,5	0,68
XII	n C_4H_9	71,8	130—1	56,64	6,02	7,01	7,68	$C_{20}H_{26}N_2O_6S$	56,85	6,20	6,63	7,58	189,5	0,82
XIII	iso-C_4H_9	71,5	125—6	57,01	6,15	6,85	7,40	$C_{20}H_{26}N_2O_6S$	56,85	6,20	6,63	7,58	180,6	0,80

*Melt with decomposition.

TABLE 3. m-Methoxy-p-alkoxybenzylpenicillins

Compound	R	Yield %	Melting point (in deg)*	Found (in %)				Empirical formula	Calculated (in %)				$[\alpha]_D^{20}$ (concn. 0,87, water)	R_f
				C	H	N	S		C	H	N	S		
XIV	CH_3	62,3	84—5	54,68	5,53	7,37	8,40	$C_{18}H_{22}N_2O_6S$	54,80	5,62	7,15	8,12	227	0,56
XV	C_2H_5	62,8	109—10	55,63	6,30	6,84	7,69	$C_{19}H_{24}N_2O_6S$	55,86	5,92	6,85	7,85	239	0,73
XVI	n-C_3H_7	63,3	89—90	56,79	6,40	7 00	7,26	$C_{20}H_{26}N_2O_6S$	56,85	6,20	6,63	7,58	189	0,64
XVII	iso-C_3H_7	58,9	76—7	56,48	6,12	6,41	7,73	$C_{20}H_{26}N_2O_6S$	56,85	6,20	6,63	7,58	189	0,74
XVIII	n-C_4H_9	60,5	124—5	57,45	6,21	6,15	7,08	$C_{21}H_{28}N_2O_6S$	57,78	6,46	6,41	7,34	207,7	0,76
XIX	iso-C_4H_9	59,3	79—80	58,05	6,74	6,08	6,99	$C_{21}H_{28}N_2O_6S$	57,78	6,46	6,41	7,34	189	0,69

*Melt with decomposition

The groups of penicillins noted above were prepared by the acid chloride method [2, 4].

The reaction for preparing penicillins I-IV and VIII-XIII was carried out by reacting sodium 6-aminopenicillinate (6-AP) with the acid chlorides of the respective benzoic acids in an aqueous acetone medium in the presence of sodium bicarbonate. Penicillins V-VII and XIV-XIX were obtained by reacting 6-AP with the acid chlorides of the respective phenylacetic acids in absolute acetone in the presence of triethylamine.

The starting acids and their acid chlorides had already been prepared by the methods described in [5, 6].

The structure of the penicillins obtained was confirmed by their IR spectra, thin layer chromatography, and elemental analysis.

The IR spectra were run on a UR-10 spectrophotometer as mineral oil mulls. The characteristic absorption bands of the carbonyl in the β-lactam ring (1765 to 1790 cm^{-1}), the carbonyl in the amide group (1640 to 1660 cm^{-1}), and the carbonyl in the carboxyl group (1710 to 1725 cm^{-1}), plus those of the benzene ring (1600 to 1615 cm^{-1}) [7] were detected.

Thin layer chromatography was carried out by a known method [8]. Ordinary pharmaceutical talc was used as the substrate. An n-butanol-ether-acetone-water (14:4.6:4.5:5) eluting system was employed. The chromatogram was developed for 3 to 4 h. The chromatograms were treated with ammonia vapors for 30 min, and developed by using the reaction between the penicillins and iodine subsequent to splitting the β-lactam ring.

The optical activity was determined on a circular polarimeter; all the penicillins obtained were optically active.

The penicillins were prepared as their sodium salts in order to investigate their biological properties.

Their antibacterial activity in relation to various organisms was determined by the two-fold series dilutions method. The minimum bacteriostatic concentration of the sodium salt of benzylpenicillin was determined in each experiment as a control. All the preparations were used in equivalent amounts with respect to benzylpenicillin so that a comparative evaluation of the antibacterial activity of the penicillins with various side chains could be made versus benzylpenicillin. The experiments were repeated five times. The acute toxicity was determined on mice using a single intravenous injection of the substances we studied. The maximum bearable dose was determined.

The antibacterial spectrum of phenylpenicillin and o-, m-, and p-methoxy-substituted phenyl- and benzylpenicillins did not differ from the spectrum of benzylpenicillin (Table 4). The compounds are active against gram-positive microorganisms and inactive against gram-negative ones. m-Methoxyphenylpenicillin (III) is the most active. No regularity with respect to the activity of the various isomers is observed when the antibacterial activity of methoxy substituted phenyl- and benzylpenicillins are compared. Apart from m-methoxyphenylpenicillin, the benzylpenicillins are basically more active than their phenyl analogs. Only m-methoxyphenylpenicillin was active in somewhat less concentration than benzylpenicillin with respect to the six resistant staphylococcus strains that were tested. The other compounds were inactive. Since m-methoxyphenylpenicillin was the most active of all the compounds tested, m-methoxy-p-alkoxyphenylpenicillins were synthesized for the purpose of studying the

TABLE 4. Antibacterial Spectrum of Alkoxyphenyl- and Benzylpenicillins

Compound	Minimum bacteriostatic concentration (in μg/ml)													
	Strept. pyogenes	Staph. aureus 209 p	Staph. albus	Staph. aureus (six resistant strains)	B. subtilis ATCC 6633	B. megaterium	E. typhi	Sh. dysenteriae Flexneri	E. coli O-55	Prot. vulgaris	Ps. aeruginosa	Alc. fecalis	Vibrion Metsenikoff	Mys. smegmatis
I	0,048	0,19	0,09	31,2—250	3,9	3,9	62,5	125	>500	500	>500	>500	3,9	>500
II	31,2	62,5	125	>500	500	250	>500	>500	>500	>500	>500	>500	>500	>500
III	0,048	0,048	0,024	15,6—250	1,95	7,8	125	125	>500	>500	>500	>500	3,9	>500
IV	15,6	15,6	7,8	>500	500	500	>500	>500	>500	>500	>500	>500	500	>500
V	0,12	0,24	0,12	>500	0,9	7,8	62,5	250	>500	>500	>500	>500	31,2	>500
VI	0,06	0,24	0,24	250—500	1,95	15,6	62,5	500	>500	>500	>500	>500	250	>500
VII	0,12	0,9	0,9	>500	3,9	150	125	>500	>500	>500	>500	>500	>500	>500
VIII	0,39	0,78	0,39	62,5—500	7,8	31,2	125	500	>500	>500	>500	>500	31,2	>500
IX	0,78	3,12	1,56	62,5—500	15,6	125	250	>500	>500	>500	>500	>500	31,2	>500
X	3,12	6,25	6,25	250—500	62,5	500	250	>500	>500	>500	>500	>500	250	>500
XI	3,12	6,25	12,5	500—500	125	500	500	>500	>500	>500	>500	>500	250	>500
XII	0,78	3,12	1,56	125—500	7,8	125	250	>500	>500	>500	>500	>500	62,5	>500
XIII	0,19	0,78	0,39	62,5—500	3,9	31,2	125	>500	>500	>500	>500	>500	15,6	>500
XIV	3,9	15,6	15,6	500—500	31,2	>500	>500	>500	>500	>500	>500	250	500	>500
XV	3,9	15,6	15,6	500—500	125	>500	>500	>500	>500	>500	>500	>500	500	>500
XVI	0,12	0,48	0,48	62,5—500	7,8	62,5	250	>500	>500	>500	>500	>500	62,5	>500
XVII	0,24	0,9	0,9	500—500	7,8	125	250	>500	>500	>500	>500	>500	125	>500
XVIII	3,9	15,6	15,6	500—500	250	>500	>500	>500	>500	>500	>500	>500	>500	>500
XIX	62,5	31,2	15,6	500—>500	>250	>500	>500	>500	>500	>500	>500	>500	>500	>500
Benzylpenicillin	0,003	0,012	0,006	31,2—500	0,048	0,19	0,39	3,12	31,2	1,56	>500	125	0,78	>500

relationship of the antibacterial activity both from the standpoint of the quantity of the alkoxy groups and from the size of the alkyl groups. It was established that these penicillins possess a low antibacterial activity. They are also not active against resistant staphylococcus strains. Consequently, the introduction of a second alkoxy group into the para position leads in all cases to a decrease in activity.

Benzylpenicillins which contain those same alkoxy groups in the meta and para position of the benzyl ring are inactive with the exception of the compounds containing methoxy and propoxy or methoxy and isopropoxy groups.

The size of the alkyl groups is reflected in different ways in the substituted phenyl- and benzylpenicillins. Although penylpenicillins containing methoxy and isobutoxy groups are the most active, the introduction of these same groups into benzylpenicillins (XIV and XIX) sharply lowers the activity. All of the penicillins we studied had low toxicity. Their tolerance is the same and does not differ from the tolerance of the sodium salt of benzylpenicillin (the maximum tolerable dose is 1500 to 2000 μg/kg).

EXPERIMENTAL

m-Methoxy-p-alkoxyphenylpenicillins (VIII-XIII). To a solution of 0.02 mole of 6-AP and 5 g of sodium bicarbonate in a mixture of 90 ml of water and 50 ml of acetone was added over a 15 min period with stirring and cooling to 0°C 0.02 mole of m-methoxy-p-alkoxybenzoyl chloride. The reaction mixture was stirred for another hour with cooking and for 4 h at room temperature, then it was extracted twice with ether. The ether extract was discarded and the aqueous layer was cooled to 5 to 7°C, 100 ml of ether was added to it, and it was acidified with 1 N hydrochloric acid with stirring to pH 2.0. The ether layer was separated. The acidified aqueous layer was additionally extracted with ether. The combined ether extracts were washed with ice water and shaken with anhydrous sodium sulfate. The penicillin was recovered from the ether extract by fractionally adding an 8% aqueous sodium bicarbonate solution to it until the aqueous layer had a pH of 6.5 to 7.0. The latter was separated, extracted with ether, and evaporated in a vacuum desiccator over phosphorus pentoxide. The crystalline residue obtained was triturated with absolute ether. A small amount of the sodium salt was converted again into the penicillin-acid, the ether extract was evaporated under reduced pressure, and the remainder was crystallized from petroleum ether.

Methoxyphenylpenicillins (I to IV) were similarly obtained.

m-Methoxy-p-alkoxybenzylpenicillins (XIV to XIX). A mixture of 0.05 mole of 6-AP and 100 ml of absolute acetone was cooled to 0°C, 4.5 ml of triethylamine was added, the mixture was stirred for 10 min, and 0.06 mole of m-methoxy-p-alkoxyphenylacetyl chloride in 60 ml of absolute acetone and 13.5 ml of triethylamine were added simultaneously over a 30 min period. The mixture was stirred another 2 h at room temperature, filtered, and the residue was washed with absolute acetone. The combined filtrates were cooled to 7°C, diluted with twice their volume of ice water, and extracted with ether. The water layer was separated, half of its volume of ether was added to it, and it was acidified to pH 2.0 with 5 N sulfuric acid with stirring. The ether layer was separated. The acidified aqueous layer was extracted with ether twice more, the combined ether extracts were treated with anhydrous sodium sulfate and carbon, filtered, and shaken with a 3% aqueous sodium bicarbonate solution, gradually bringing the aqueous layer to pH 7.0. The latter was extracted with ether and evaporated in the cold at a reduced pressure. The crystalline residue of the sodium salt was triturated with absolute ether. It was again converted to the penicillin-acid, which was crystallized from petroleum ether, in order to determine its physicochemical constants.

The sodium salts of the penicillins were dissolved in aqueous acetone (1:5) and precipitated with absolute acetone with cooling in order to purify them.

Methoxybenzylpenicillins (V to VII) were similarly prepared.

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