INVESTIGATIONS IN THE FIELD OF SEMISYNTHETIC PENICILLINS. XIV. 1-(ALKOXYPHENYL)CYCLOPENTYL-1-PENICILLINS

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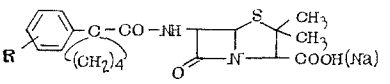
UDC 615.334

The present work is a continuation of investigations on the synthesis and study of the properties of certain new penicillins of the cycloaliphatic series [1] of general formula



R = CH_3O to C_4H_9O.

Nitriles of 1-(o, m, p-alkoxyphenyl)cyclopentane 1-carboxylic acids were used as starting materials for the synthesis of these penicillins. The nitriles were obtained by the condensation of the appropriate alkoxyphenylacetonitriles with 1,4-dibromobutane using powdered sodium hydroxide [2] at a temperature of 90-120°C. Of the nitriles obtained the phenyl, p-alkoxy- and m-methoxyphenylcyclopentyl derivatives have been described in the literature [2, 3]. The synthesis of these compounds was reproduced with the aim of completing the series of alkoxyphenylcyclopentylpenicillins.

Hydrolysis of the initial nitriles into 1-phenyl- and 1-(o, m, p-alkoxyphenyl)cyclopentane 1-phenyl- and 1-(o, m, p-alkoxyphenyl)cyclopentane 1-carboxylic acids was effected by boiling them with potassium hydroxide in a medium of diethylene glycol for 6-8 h in contrast to the method described in the literature [4], the authors of which carried out the reaction in an autoclave at 130-140°C for 12 h and used 20% potassium hydroxide solution.

To obtain 1-phenylcyclopentyl-1-penicillin the method [5] used acylation of 6-aminopenicillanic acid (6-APA) with the acid chloride of 1-phenylcyclopentane 1-carboxylic acid [6], which was obtained by heating the initial acid for 6 h with thionyl chloride in a medium of absolute benzene [6].

For the preparation of penicillins with alkoxy substituents in the benzene ring the acylation of 6-APA used the mixed anhydrides of the corresponding acids [7].

The structures of the obtained penicillins were confirmed by elemental analysis, and by IR spectra, which showed the presence of the β-lactam and thiazolidine rings (1765-1790 cm^{-1}), amide (1640-1660 cm^{-1}), and carboxyl (1710-1725 cm^{-1}) groups, and the benzene ring (1600-1615 cm^{-1}). The purity of preparations was checked by thin layer chromatography [8] and bromocoulometric titration [9]. Penicillins were characterized as acids (Table 1) and tested as the sodium salts. Antibacterial activity, the extent of hydrolysis by staphylococcal β-lactamase, and acid stability of the penicillin, were determined by methods described previously [10, 11].

The alkoxyphenylcyclopentylpenicillins were active in relation to gram-positive microorganisms (Table 2). They did not possess marked sensitivity in relation to gram-negative microorganisms. Some of them were more active than benzylpenicillin in relation to Vibrio Metschnicoff. The unsubstituted derivative possessed more marked antibacterial properties. The obtained penicillins were somewhat more active in comparison with benzylpenicillin in relation to resistant staphylococci producing penicillinase (see Table 2). Thus, benzylpenicillin suppresses the growth of 4 clinically resistant strains of staphylococci at concentrations of 31.2-2000 μg/ml, but alkoxyphenylcyclopentylpenicillins suppress the growth of these same cultures at concentrations of 7.8-500 μg/ml. On increasing the bacterial loading of a resistant staphylococcus (strain No. 9) 100 fold (ratio of seeding dose 10^5 to 10^3 microbial bodies) the bacteriostatic concentration of benzylpenicillin was

A. L. Mndzhoyan Institute of Fine Organic Chemistry, Academy of Sciences of the Armenian SSR, Erevan. Translated from Khimiko-farmatsevticheskii Zhurnal, Vol. 14, No. 3, pp. 53-59, March, 1980. Original article submitted April 2, 1979.

0091-150X/80/1403-0179$07.50

TABLE 1. Properties of 1-Alkoxyphenylcyclopentyl-1-penicillins

Compound	R	Yield, %	mp °C*	Found, %		Empirical formula	Calculated, %		R_f†
				N	S		N	S	
I	H‡	72,5	114—5	7,07	8,12	$C_{20}H_{24}N_2O_4S$	7,21	8,24	0,72
II	o-CH_3O	69,3	132—3	6,46	7,39	$C_{21}H_{26}N_2O_5S$	6,69	7,66	0,68
III	o-C_2H_5O	73,6	114—6	6,21	7,35	$C_{22}H_{28}N_2O_5S$	6,48	7,41	0,64
IV	o-C_3H_7O	71,8	88—90	6,55	7,45	$C_{23}H_{30}N_2O_5S$	6,27	7,18	0,66
V	o-iso-C_3H_7O	64,2	123—5	6,56	7,52	$C_{23}H_{30}N_2O_5S$	6,27	7,18	0,63
VI	o-C_4H_9O	63,6	84—6	6,28	7,19	$C_{24}H_{32}N_2O_5S$	6,08	6,96	0,70
VII	m-CH_3O	71,5	109—10	6,40	7,46	$C_{21}H_{26}N_2O_5S$	6,69	7,66	0,66
VIII	m-C_2H_5O	60,9	99—101	6,28	7,23	$C_{22}H_{28}N_2O_5S$	6,48	7,41	0,58
IX	m-C_3H_7O	65,4	105—7	6,37	7,34	$C_{23}H_{30}N_2O_5S$	6,27	7,18	0,74
X	m-iso C_3H_7O	72,5	84—6	6,15	7,48	$C_{23}H_{30}N_2O_5S$	6,27	7,18	0,67
XI	m-C_4H_9O	70,1	102—4	6,22	6,77	$C_{24}H_{32}N_2O_5S$	6,08	6,96	0,61
XII	p-CH_3O	69,7	98,9	6,43	7,76	$C_{22}H_{28}N_2O_5S$	6,69	7,66	0,69
XIII	p-C_2H_5O	64,8	128—30	6,23	7,70	$C_{22}H_{28}N_2O_5S$	6,48	7,41	0,75
XIV	p-C_3H_7O	65,6	112—4	6,20	6,95	$C_{23}H_{30}N_2O_5S$	6,27	7,18	0,72
XV	p-iso C_3H_7O	66,2	106—8	6,54	7,25	$C_{23}H_{30}N_2O_5S$	6,27	7,18	0,71
XVI	p-C_4H_9O	62,4	118—20	6,30	6,75	$C_{24}H_{32}N_2O_5S$	6,08	6,96	0,73

*Melting with decomposition.

†System butanol—water—acetone—ether (14:4.5:4.5:5).

‡Described in the literature [5].

raised 48-fold but the bacteriostatic concentrations of penicillins (I, XII-XVI) were increased only 4-8-fold. The inactivation of the obtained penicillins by staphylococcal β-lactamase has been checked. All the penicillins appeared resistant to its action (Table 3). Phenylcyclopentylpenicillin had 100% stability in the first hour of inactivation. The introduction of alkoxy radicals into various positions of the benzene ring led to a reduction in stability in comparison with the unsubstituted derivative. The somewhat more stable derivatives were mainly substituted in the meta position. At this position replacement of an n-propoxy radical by the radical of iso structure was accompanied by a fall in the stability of the penicillin towards β-lactamase. The analogous replacement at the ortho and para positions was not accompanied by a change in stability. In the last hours of inactivation the hydrolysis rate of the penicillin was somewhat increased. However their stability towards the enzyme survived into the fourth hour of inactivation. The inactivation of penicillins (XIV, XV) during 4 h are shown as examples (see Fig. 1).

The penicillins were more acid stable than benzylpenicillin, the decomposition half-life of which under the conditions of our experiment was 2.1 min. The decomposition half life of phenylcyclopentylpenicillin was 26 min. The introduction of alkoxy radicals led mainly to an increase in the acid stability of the unsubstituted derivative. There are penicillins the decomposition half lives of which were 160-287 min (see Table 3).

All the alkoxy substituted phenylcyclopentylpenicillins had low toxicity. They were well tolerated on single intravenous injection to white mice at doses from 1500-2500 mg/kg. The unsubstituted derivative was more toxic (see Table 3). The inactivation kinetics of all the penicillins were tested for 4 h. The inactivation of compounds (XIV, XV) are given as examples. The studied group of penicillins had low toxicity, were acid stable, possessed activity in relation to gram-positive microorganisms, and had stability towards the action of staphylococcal β-lactamase.

EXPERIMENTAL

1-(o, m, p-Alkoxyphenyl)-1-cyanocyclopentanes. A mixture of powdered sodium hydroxide (2.0 g; 0.5 mole) and alkoxyphenylacetonitrile (0.14 mole) was heated to 35 to 45°C. 1,4-Dibromobutane (32 g; 0.14 mole) was added with stirring at this temperature. After the addition of all the 1,4-dibromobutane the temperature was raised to 110-120°C and the reaction mixture was maintained at this temperature for 10-12 h. After cooling, water (100 ml) and ether (150 ml) were added, the ether layer was separated, the water layer was extracted with ether, and added to the main product. The ether extract was washed with water and dried over calcined sodium sulfate. After distilling off the ether the residue was distilled in vacuum (Table 4).

1-(o, m, p-Alkoxyphenyl)cyclopentane 1-Carboxylic Acids. A mixture of 1-(o, m, p-alkoxyphenyl)-1-cyanocyclopentane (0.1 mole) and potassium hydroxide (15.6 g; 0.28 mole) in diethylene glycol (150 ml) was boiled for 6 h after which the cooled mixture was poured into water (150 ml) and extracted twice with ether. The aqueous layer was acidified with dilute (1:1) hydrochloric acid until an acid reaction was given to Congo. The acid which had precipitated was filtered off after 6 h, and washed with water to neutral reaction (Table 5).

TABLE 2. Spectrum of Antibacterial Action of Alkoxyphenylcyclopentylpenicillins

Compound	R*	Test microbe												
		Strept. pyogenes	Staph. albus	Staph. aureus 209P	Staph. aureous 209 P (adapted to benzylpenicillin)	Staph. aureus (4 clinical strains)	B. subtilis ATSS 6633	E. typhi	Sh. dysenteriae flexneri	E. coli 0-55	Prot. vulgaris	Ps. aeruginosa	Vibrion Metschnicoff	Myco. Smegmatis
		minimum bacteriostatic concentration μg/ml												
I	H	0,012	0,048	0,48	>500	7,8—250	0,09	62,5	125	500	250	>500	0,24	>500
II	o-CH_3O	0,78	0,9	0,9	>500	62,5—250	3,9	62,5	62,5	250	500	>500	—	>500
III	o-C_2H_5O	0,78	0,39	0,78	>500	62,5—250	7,8	62,5	125	500	500	>500	0,09	>500
IV	o-C_3H_7O	0,39	0,39	0,19	>500	125—250	15,6	62,5	125	125	500	>500	0,9	>500
V	o-isoC_3H_7O	0,78	0,39	0,39	>500	125—250	7,8	125	250	500	500	>500	0,9	>500
VI	o-C_4H_9O	0,39	0,19	0,39	>500	125—250	15,6	125	250	500	500	>500	0,9	>500
VII	m-CH_3O	0,39	0,39	0,39	>500	125—500	15,6	—	125	250	125	>500	1,56	>500
VIII	m-C_2H_5O	0,39	0,19	0,39	>500	250—500	0,9	31,2	125	250	250	>500	3,9	>500
IX	m-C_3H_7O	0,39	0,19	0,39	>500	125—500	0,9	15,6	62,5	250	125	>500	3,9	>500
X	m-iso-C_3H_7O	0,19	0,19	0,39	>500	250—500	7,8	15,6	62,5	250	250	>500	3,9	>500
XI	m-C_4H_9O	0,39	0,19	0,39	—	125—250	1,56	—	125	125	125	—	15,6	>500
XII	p-CH_3O	0,048	0,19	0,19	—	31,2—250	0,24	7,8	31,2	62,5	62,5	>500	0,48	>500
XIII	p-C_2H_5O	0,048	0,09	0,19	—	62,5—250	0,24	7,8	31,2	62,5	62,5	>500	0,19	>500
XIV	p-C_3H_7O	0,048	0,09	0,39	—	62,5—250	0,24	7,8	31,2	125	62,5	>500	0,9	>500
XV	p-iso-C_3H_7O	0,09	0,19	0,19	—	62,5—250	0,24	7,8	31,2	125	62,5	>500	0,48	>500
XVI	p-C_4H_9O	0,048	0,19	0,39	—	125—500	0,24	15,6	62,5	125	125	>500	0,48	>500
Benzylpenicillin		0,003	0,006	0,012	>500	31,2—2000	0,048	0,39	3,12	31,2	1,56	>500	0,78	>500

*R see general formula.

TABLE 3. Biological Characteristics of 1-(Alkoxyphenyl)cyclopentyl-1-penicillins

Compound	No. of units of penicillins inactivated under the action of 1 unit staphylococcal β-lactamase	$\tau_{1/2}$, min	MPD mg/kg
I	0	26,0	500
II	28,8 (11÷46,6)	187,5	2000
III	20,5 (0,7÷40,3)	102,1	1000
IV	20,45 (10,93÷29,97)	14,9	1500
V	12 (5,8÷18,2)	10,3	1500
VI	16,6 (7÷26,2)	14,8	1000
VII	14,2 (9,7÷18,7)	287,8	2500
VIII	10,0 (3,3÷16,7)	189,4	1500
IX	10,6 (1,9÷19,3)	222,0	1500
X	27,5 (13,3÷41,7)	25,9	1500
XI	11,2 (1÷21,4)	277,7	2000
XII	40,3 (28,7÷51,9)	163,0	2000
XIII	24,9 (1÷47,0)	99,2	1500
XIV	10,4 (1,8÷19,0)	88,9	1500
XV	9,4 (1÷17,8)	160,0	1500
XVI	23,3 (15,0÷31,6)	30,5	1500
Benzylpenicillin	60,0	2,1	1500

Note. The limits of variation are given in parentheses. Conditions were pH 1.3, 37°C.

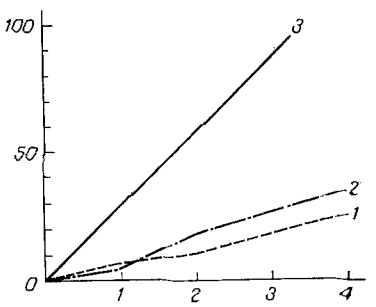


Fig. 1. Inactivation kinetics of penicillins under the action of staphylococcal β-lactamase. Inactivation time (h) is given on the abscissa and the extent of inactivation of the penicillin (%) on the ordinate. Inactivation curves are for: 1) compound (XIV); 2) compound (XV); 3) benzylpenicillin.

TABLE 4. Properties of Nitriles of 1-Alkoxyphenylcyclopentane 1-Carboxylic Acids

Compound	R	Yield, %	bp, °C (mm)	d_4^{20}	n_D^{20}	Found, %			Empirical formula	Calculated, %		
						C	H	N		C	H	N
Ia	H	75,6	135—40 (10)	1,0298	1,5322	84,35	7,82	7,98	$C_{12}H_{13}N$	84,16	7,65	8,18
IIa	o-CH_3O*	48,2	145—8 (3)	—	—	77,21	7,75	7,15	$C_{13}H_{15}NO$	77,53	7,50	6,95
IIIa	o-C_2H_5O	50,6	155—8 (3)	1,0594	1,5266	77,86	8,26	6,81	$C_{14}H_{17}NO$	78,10	7,95	6,50
IVa	o-C_3H_7O	57,1	175—8 (3)	1,0508	1,5280	78,81	8,56	6,25	$C_{15}H_{19}NO$	78,56	8,35	6,10
Va	o-iso C_3H_7O	61,2	170—3 (3)	1,0595	1,5272	78,25	8,62	6,34	$C_{15}H_{19}NO$	78,56	8,35	6,10
VIa	o-C_4H_9O	63,1	200—3 (3)	1,0534	1,5218	78,85	8,74	6,05	$C_{16}H_{21}NO$	78,97	8,69	5,75
VIIa	m-CH_3O†	63,5	168—71 (3)	1,0959	1,5348	77,81	7,33	6,70	$C_{13}H_{15}NO$	77,53	7,50	6,95
VIIIa	m-C_2H_5O	58,9	195—8 (3)	1,0572	1,5362	78,35	8,20	6,75	$C_{14}H_{17}NO$	78,10	7,95	6,50
IXa	m-C_3H_7O	65,2	186—9 (3)	1,0614	1,5275	78,21	8,15	6,25	$C_{15}H_{19}NO$	78,56	8,35	6,10
Xa	m-iso-C_3H_7O	50,1	174—7 (3)	1,0016	1,5220	78,75	8,65	5,95	$C_{15}H_{19}NO$	78,56	8,35	6,10
XIa	m-C_4H_9O	70,4	189—93 (3)	1,0210	1,5213	78,54	8,50	7,45	$C_{16}H_{21}NO$	78,97	8,69	7,75
XIIa	p-CH_3O ‡	70,7	135—9 (3)	1,0862	1,5360	77,52	7,60	6,72	$C_{13}H_{15}NO$	77,53	7,50	6,95
XIIIa	p-C_2H_5O‡	73,2	158—61 (3)	1,0548	1,5291	78,42	8,29	6,78	$C_{14}H_{17}NO$	78,10	7,95	6,50
XIVa	p-C_3H_7O ‡	73,6	178—81 (3)	1,0417	1,5250	78,54	8,10	5,82	$C_{15}H_{19}NO$	78,56	8,35	6,10
XVa	p-iso-C_3H_7O ‡	72,5	156—9 (3)	1,0408	1,5240	78,76	8,46	6,00	$C_{15}H_{19}NO$	78,56	8,35	6,10
XVIa	p-C_4H_9O ‡	74,2	188—92 (3)	1,0248	1,5220	79,11	8,47	6,06	$C_{16}H_{21}NO$	78,97	8,69	5,75

*Viscous substance.

†Described in the literature [3].

‡Described in the literature [2].

TABLE 5. Properties of 1-Alkoxyphenylcyclopentane 1-Carboxylic Acids

Compound	R	Yield, %	mp °C	Found, %		Empirical formula	Calculated, %	
				C	H		C	H
Ib	H	94,9	157—8 †	75,56	7,25	$C_{12}H_{14}O_2$	75,76	7,41
IIb*	o-CH_3O	62,5	—	71,05	7,56	$C_{13}H_{16}O_3$	70,88	7,32
IIIb*	o-C_2H_5O	87,6	—	71,56	8,02	$C_{14}H_{18}O_3$	71,76	7,74
IVb*	o-C_3H_7O	80,1	—	72,85	8,42	$C_{15}H_{20}O_3$	72,55	8,10
Vb*	o-iso C_3H_7O	82,3	—	72,25	7,87	$C_{15}H_{20}O_3$	72,55	8,10
VIb*	o-C_4H_9O	84,1	—	73,15	8,60	$C_{16}H_{22}O_3$	73,21	8,45
VIIb	m-CH_3O	62,5	118—9	70—75	7,21	$C_{13}H_{16}O_3$	70,88	7,32
VIIIb	m-C_2H_5O	72,9	125—6	72,10	7,54	$C_{14}H_{18}O_3$	71,76	7,74
IXb	m-C_3H_7O	78,2	129—30	72,84	8,36	$C_{15}H_{20}O_3$	72,55	8,10
Xb	m-iso C_3H_7O	78,9	99—100	72,56	8,32	$C_{15}H_{20}O_3$	72,55	8,10
XIb	m-C_4H_9O	63,1	105—6	73,54	8,15	$C_{16}H_{22}O_3$	73,21	8,45
XIIb	m-CH_3O	66,6	140—1 †	70,98	7,52	$C_{13}H_{16}O_3$	70,88	7,32
XIIIb	p-C_2H_5O	75,4	150—1	71,85	8,05	$C_{14}H_{18}O_3$	71,76	7,74
XIVb	p-C_3H_7O	76,1	128—9	72,72	7,91	$C_{15}H_{20}O_3$	72,55	8,10
XVb	p-iso C_3H_7O	86,1	169—170	72,45	7,85	$C_{15}H_{20}O_3$	72,55	8,10
XVIb	p-C_4H_9O	79,0	115—6	72,95	8,65	$C_{16}H_{22}O_3$	73,21	8,45

*Viscous substance.

†Described in the literature [3].

1-(o, m, p-Alkoxyphenyl)cyclopentyl-1-penicillins. To a solution of 1-(o, m, p-alkoxyphenyl)cyclopentane 1-carboxylic acid (0.02 mole) in absolute acetone (60 ml) was added with stirring and cooling to 0°C triethylamine (2.4 g; 0.024 mole) in absolute acetone (40 ml) and ethyl chloroformate (3 g; 0.028 mole) in absolute acetone (20 ml). The mixture was stirred for 30 min at 0°C and 2 h at room temperature and then filtered. The filtrate was added to a mixture containing 6-APA (5.6 g; 0.026 mole) in acetone (120 ml) and 2.5% sodium bicarbonate solution (100 ml). The mixture was stirred for 4 h, water (100 ml) added, and the greater part of the acetone distilled off in vacuum with cooling. The residue was extracted with ether and the aqueous layer acidified with 1 N hydrochloric acid to pH 2.0 with cooling and stirring. The isolated penicillin derivatives were extracted with ethyl acetate. The extracts were combined, washed with water, and shaken with active carbon (5 g) and anhydrous sodium sulfate for 20 min. The ethyl acetate solution was filtered off, a portion was left for isolation of the acid by crystallization from petroleum ether, and the remainder was treated with 8% sodium bicarbonate solution to pH 7.0 in the aqueous layer. The aqueous layer was separated, washed with ether, and lyophilized (see Table 1).

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