SYNTHESIS OF 5-SUBSTITUTED N(1)- AND N(2)-TETRAZOLYLACETIC ACIDS AND THEIR BIOLOGICAL PROPERTIES

M. V. Aleksanyan, M. S. Kramer, S. G. Akopyan, R. V. Agababyan, Yu. Z. Ter-Zakharyan, L. G. Akopyan, G. M. Paronikyan, and Sh. L. Mndzhoyan

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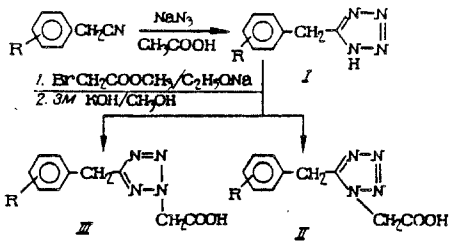
Tetrazole and its derivatives are of great practical interest because of their biological activity and are widely used in pharmaceutical preparations. The attention of research workers has been particularly drawn to 5-aryltetrazolylalkanoic acids as potential anti-inflammatory agents [1].

In the search for new biologically active compounds in the tetrazole series, we synthesized 5-alkoxybenzyl-1H-1- (II) and 2H-2-tetrazolylacetic acids (III) and studied their anti-inflammatory, antibacterial and mutagenic properties.



R=H (Ia, IIa, IIIa), *o*-OMe (Ib, IIb, IIIb), *m*-OMe (Ic, IIc, IIIc), p-OMe (Id, IId, IIId), *o*-OEt (Ie, IIe, IIIe), *m*-OEt (If), p-OEt (Ig, IIg, IIIg), *o*-Pr (Ih, IIh, IIIh); *m*-OPr (Ii, IIi, IIIi); p-OPr (Ij, IIj, IIIj).

For use as starting compounds, we synthesized 5-alkoxybenzyltetrazoles Ia-j by cyclization of alkoxyphenylacetonitriles [2] with sodium azide in the presence of glacial acetic acid in an *n*-butanol medium [7]. The purity and individuality of tetrazoles Ia-j was confirmed by TLC method and the structure by the elemental analysis, mass and PMR spectral data.

The mass spectra are characterized by molecular ion peaks and several characteristic fragmentary ions. The PMR spectra of compounds Ia-j show singlet signals of the methylene group protons of the benzyl moiety (δ 4.2-4.3 ppm), NH signals of the tetrazole ring (δ 4.6-5.3 ppm) and the multiplet signal of the aromatic protons in the 6.7-7.8 ppm region.

One of the methods for the preparation of 5-substituted N-tetrazolylacetic acids is the alkylation of tetrazoles, which generally results in the formation of isomeric 1,5- and 2,5-disubstituted tetrazoles, according to the nature of the substituent at C(5), the type of the alkylating agent and the reaction medium [4, 5].

The alkylation of tetrazoles Ia-j was effected by the action of methyl bromoacetate in the presence of sodium alcoholate with the formation of the intermediate esters of tetrazoleacetic acids which were hydrolyzed without prior isolation and purification by a methanolic solution of potassium hydroxide. Examination of the reaction showed that the process proceeds with the formation of a mixture of two isomeric products II and III, which could be effectively separated by fractional crystallization. As a result, one of the isomers — N(1)-tetrazolylacetic acid II — was precipitated during the course of the treatment by acidification of the aqueous medium to pH 2, while the other iso-

A. L. Mndzhoyan Institute of Fine Organic Chemistry, Academy of Sciences of the Armenian SSR, Erevan.
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TABLE 1. Properties of Tetrazoles Ia-j

Compound	Yield,%	MP, °C	R_f	Empirical formula
Ia	83,5	120—121	0,50	$C_8H_8N_4$
Ib	29,7	148—149	0,63	$C_9H_{10}N_4O$
Ic	51,6	101—102	0,51	$C_9H_{10}N_4O$
Id	76,5	153—154	0,52	$C_9H_{10}N_4O$
Ie	30,7	125—126	0,54	$C_{10}H_{12}N_4O$
If	37,7	138—139	0,5	$C_{10}H_{12}N_4O$
Ig	49,7	134—135	0,47	$C_{10}H_{12}N_4O$
Ih	22,8	108—109	0,63	$C_{11}H_{14}N_4O$
Ii	23,7	75—76	0,60	$C_{11}H_{14}N_4O$
Ij	39,8	115—116	0,55	$C_{11}H_{14}N_4O$

TABLE 2. Properties of N(1)- and N(2)-Tetrazolylacetic Acids IIa-j and IIIa-j

Compound	Yield, %	Mp, °C	R_b	M* found	Empirical formula	M calculated
IIa	51,5	173—174	0,4	218	$C_{10}H_{10}N_4O_2$	218,2
IIb	40,3	186—187	0,41	248	$C_{11}H_{12}N_4O_3$	248,2
IIc	20,16	166—167	0,37	248	$C_{11}H_{12}N_4O_3$	248,2
IId	36,3	182—183	0,40	248	$C_{11}H_{12}N_4O_3$	248,2
IIe	27,6	78—80	0,42	262	$C_{12}H_{14}N_4O_3$	262,3
IIg	25,8	141—142	0,47	262	$C_{12}H_{14}N_4O_3$	262,3
IIh	30,5	85—86	0,44	276	$C_{13}H_{16}N_4O_3$	276,5
IIi	35,1	81—82	0,49	276	$C_{13}H_{16}N_4O_3$	276,5
IIj	32,9	130—131	0,43	276	$C_{13}H_{16}N_4O_3$	276,5
IIIa	10,8	82—83	0,44	218	$C_{10}H_{10}N_4O_2$	218,2
IIIb	14,1	98—99	0,4	248	$C_{11}H_{12}N_4O_3$	248,2
IIIc	18,2	102—103	0,49	248	$C_{11}H_{12}N_4O_3$	248,2
IIId	24,2	106—107	0,48	248	$C_{11}H_{12}N_4O_3$	248,2
IIIe	13,4	132—133	0,45	262	$C_{12}H_{14}N_4O_3$	262,3
IIIg	18,6	110—111	0,46	262	$C_{12}H_{14}N_4O_3$	262,3
IIIh	21,5	67—68	0,46	276	$C_{13}H_{16}N_4O_3$	276,5
IIIi	20,2	109—110	0,45	276	$C_{13}H_{16}N_4O_3$	276,5
IIIj	20,0	90—91	0,45	276	$C_{13}H_{16}N_4O_3$	276,5

*The M^+ value is given (mass spectrometrically).

mer — N(2)-tetrazolylacetic acid III — was isolated from the ether extract. The structure of the synthesized N(1)- and N(2)-tetrazolylacetic acids IIa-j and IIIa-j was confirmed by the spectral data.

The PMR spectra of IIa-j and IIIa-j show signals of aromatic protons in the 6.6-7.3 ppm region, a singlet signal of the 5-CH_2 group in the 4.2-4.3 ppm region, and also singlet signals of protons of the 1-CH_2 and 2-CH_2 groups of isomers II, III within 5.2-5.5 ppm. The proton signals of the methylene group protons bound to the tetrazole ring at the 1-position (IIa-j) resonate in a higher field than the proton signals of the methylene group bound to the tetrazole ring at the 2-position (IIIa-j), differing by an order of magnitude of 0.15-0.25 ppm, which also agrees with the literature data [6, 8].

The investigations carried out showed that the alkylation of an unsubstituted 5-benzyltetrazole under the above described conditions preferentially gives N(1)-tetrazoleacetic acid IIa with a ~5:1 ratio of the N(1)- and N(2)-isomers. When an alkoxy group is introduced into the benzene ring, the formation of the N_2-tetrazoleacetic acid increases, but the preponderance of the N_1-isomer is retained, and the ratio of the isomers is altered to between ~3:1 and 2:1.

EXPERIMENTAL (CHEMICAL)

The mass spectra were run on an MX-1303 mass spectrometer (USSR) with direct introduction of the sample into the ion source at a temperature 20-30°C lower than the melting point of the compound studied. The PMR spectra were recorded on a "Varian T-60" spectrometer (Switzerland), using TMS as internal standard.

The course of the reaction and the individuality of the compounds was monitored by means of TLC on Silufol UV-254 plates (CSSR) in the systems: acetone-hexane, 1:1, for compounds Ia-j and chloroform—methanolacetic acid—water, 90:8.1:0.8, for compounds IIa-j and IIIa-j.

TABLE 3. Mutagenic and Lethal Action of N(1)- and N(2)-Tetrazolylacetic Acids IIa-j and IIIa-j

Compound	Dose		E. coli P678 thr⁻	
	mmoles	min	survival, %	Number of revertants per 10^6 of surviving cells
IIa	25	10	43	35±4,2
IIb	25	10	1,4	245±1,8
IIc	10	10	38	9,8±0,8
IId	25	10	0,2	826±92
IIe	25	10	1,6	252±23,5
IIg	25	10	1,8	385±41,5
IIh	25	10	80	7,5±0,6
IIi	10	10	96	7±0,8
IIj	25	10	61	8,3±,09
IIIa	10	10	93	7±0,6
IIIb	10	10	84	21±2,4
IIIc	10	10	54	9,8±1,2
IIId	10	10	34	5,32±0,6
IIIe	10	10	80	18±2
IIIg	10	10	22	6,5±0,7
IIIh	10	10	62	13±1,4
IIIi	10	10	96	100±8
IIIj	10	10	100	8,5±0,9
Control	—	—	100	7

5-Alkoxybenzyltetrazoles (Ia-j). A mixture of 0.031 mole of alkoxyphenylacetonitrile, 2.8 g (0.041 mole) of NaN_3 and 2.5 g (0.041 mole) of glacial acetic acid in 25 ml of *n*-butanol was boiled for 20-25 h. Then a further 0.6 g (0.009 mole) of NaN_3 and 1.2 g (0.009 mole) of acetic acid were added, and boiling was continued for 10-12 h. The reaction mixture was cooled, 50 ml of water was added, and the mixture was distilled under reduced pressure to the complete removal of the solvent. The aqueous layer was washed with water to remove the unreacted starting nitrile and acidified by dilute (1:1) hydrochloric acid to pH 2. The crystals that separated out were filtered off, washed with water and recrystallized from ethanol (Table 1). Mass spectrum, *m/z*. Ia: 160 (M^+), 132, 131, 117, 104, 91; Ib: 190 (M^+), 148, 147, 131, 121; Ic: 204 (M^+), 176, 175, 161, 141.

5-Alkoxybenzyl-1H-1- and 2-H-2-Tetrazolylacetic acids (IIa-j, IIIa-j). A 0.02 mole portion of tetrazole I was added to sodium alcoholate, prepared from 0.46 g (0.02 mole) of sodium in 10-15 ml of absolute ethanol. The reaction mixture was heated, and 3.2 g (0.02 mole) of methyl bromoacetate was added with stirring, and the mixture was boiled for 16 h. The mixture was filtered while hot, the ethanol was evaporated, the oily residue was dissolved in ether, and the solution was extracted with a 5% solution of $NaHCO_3$ to remove the unreacted tetrazole. The ether extract was dried over anhydrous Na_2SO_4 and the ether was evaporated off. The oily residue was dissolved in 10-15 ml of methanol, a 3 M methanolic solution of potassium hydroxide (10 ml) was added, and the mixture was boiled for 2-3 h. After cooling, water was added, and the mixture was acidified with dilute (1:1) hydrochloric acid to pH 2. The crystals of N(1)-tetrazolylacetic acid II that separated out were filtered off and recrystallized from ethanol or from butanol. The aqueous filtrate was extracted with ether, the extract was dried over anhydrous Na_2SO_4 and the ether was evaporated. The residue — N(2)-tetrazolylacetic acid III — was crystallized by grinding with hexane or petroleum ether (Table 2). PMR spectrum (in acetone), δ, ppm IIa: 4.32 (2H, s, 5-CH_2), 5.26 (2H, s, 1-CH_2), 7.25 (5H, s, C_6H_5); IIIa: 4.5 (2H, s, 5CH_2), 5.4 (2H, s, 2-CH_2), 7.25 (5H, s, C_6H_5); IIb: 3.76 (3H, s, OCH_3), 4.2 (2H, s, 5-CH_2), 5.26 (2H, s, 1-CH_2), 6.66-7.26 (4H, m, C_6H_4); IIIc: 3.7 (3H, s, OCH_3), 4.13 (2H, s, 5-CH_2), 5.43 (2H, s, 2-CH_2), 6.66-7.2 (4H, m, C_6H_4).

EXPERIMENTAL (BIOLOGICAL)

The antimicrobial action of compounds IIa-j and IIIa-j was studied by the generally accepted method of double serial dilutions in a meat-peptone bouillon (pH 7.2-7.4) in relation to *Staphylococcus aureus* 209 P and dysentery *Flexner Bacillus* 6858 for a microbial load of $2 \cdot 10^6$ microbial bodies in 1 ml of medium. The minimal inhibiting concentration of growth (MIC) was established.

Some of the compounds studied have weak antibacterial activity. Thus, a difference is noted in the value of MIC according to the structure of the compounds. The values of MIC decrease depending on the position of the alkoxyl group in the benzene ring; thus, the MIC of 5-(*m*-methoxybenzyl)- and 5-(*m*-propoxybenzyl)-N(1)- and N(2)-tetrazolylacetic acids (IIc, IIIc, IIi, IIIi) is 0.62-1.25 μg/ml, while the MIC of *o*- and *m*-methoxy and propoxybenzyl derivatives (IIb, d, e, h, IIIb, d, e, h) is equal or higher than 5 mg/ml. The site of the acid residue at the nitrogen atom in the tetrazole ring does not influence the value of MIC.

The investigation of the anti-inflammatory properties of tetrazolylacetic acids IIa-d and IIIa-d on a model of carragheen induced edema of the rat paw showed that these compounds do not exhibit anti-inflammatory activity.

The mutagenic action of N(1)- and N(2)-tetrazolylacetic acids IIa-j and IIIa-j was studied by the dose-effect method on a biochemical mutant of *Escherichia coli* P-678 Hr. The activity of the compounds was determined according to the frequency of incidence of reciprocal mutations (revertants) from an auxotrophic and prototrophic state according to a locus controlling the synthesis of threonine. The spontaneously occurring mutations served as control [3].

The data in Table 3 show that N(2)-tetrazolylacetic acids IIIb, d, e, g have low activity, inducing 3-9 times more mutations than the control. The N(1)-tetrazolylacetic acids IIb, d, e, g have pronounced mutagenic activity and induce 30-118 times more mutations than the control.

Thus, among the tetrazolylacetic acids studied compounds with variable mutagenic activity were discovered, the most active being the N(1)-tetrazolylacetic acids with methoxy and ethoxy-substituents in the ortho- and para-positions of the benzene ring.

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