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Several 2-(1-acetoxy-3-substituted carbamido)-5-phenyltetrazoles were synthesized as possible antiinflammatory agents. Nmr spectroscopy was used as a tool in assigning the structure of the positional isomers of these substituted phenyltetrazoles. Compounds possessing antiinflammatory activity (100 mg./kg., *i.p.*) provided 11-41% protection against carrageenin-induced edema in rats. Seven of these substituted phenyltetrazoles possessed antiproteolytic activity and the *in vitro* inhibition of trypsin activity during hydrolysis of bovine serum albumin ranged from 9-66%.

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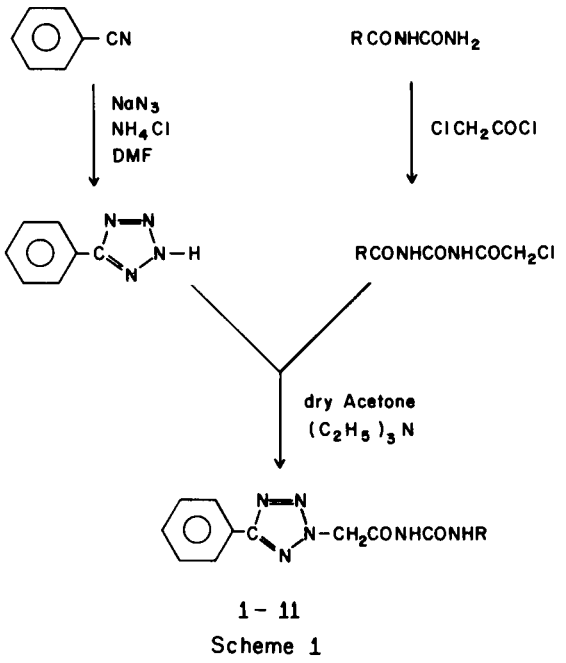
The effectiveness of 5-amino-1-phenyl tetrazole in patients with rheumatoid arthritis, who had previously failed to respond to phenylbutazone or indomethacin (1), drew interest towards synthesis and evaluation of other tetrazoles as possible nonsteroidal antiinflammatory agents. Earlier studies reported the synthesis and antiinflammatory activity of certain 3-(5-aryl-2-tetrazolyl)propionic acids and their amides (2), 5-aryltetrazolyl-2-acetamides (3), 5-(2-anilinophenyl)tetrazoles (4) and 1-substituted-3-(5-tetrazolylmethyl)indoles (5). Some of these compounds were found to show significant antiinflammatory activity when tested orally in rats against carrageenin-induced edema. In addition, the effects of substituting tetrazole moiety for carboxyl group have been discussed in two series of antiinflammatory phenoxyacetic acids (6). These observations prompted synthesis and evaluation of 2,5-disubstituted tetrazoles as possible nonsteroidal anti-inflammatory agents.

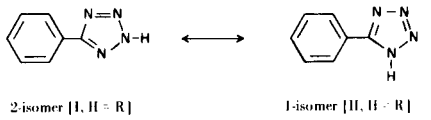
Various 2-(1-acetoxy-3-substituted carbamido)-5-phenyl tetrazoles were synthesized according to the steps outlined in Scheme 1 where *N*-substitution on the 5-phenyl tetrazole can result in the formation of two isomers. The substitution can thus result in either at 1 or 2 position. This is due to the fact that the tetrazole itself can exist in two tautomeric forms (Figure 1). Earlier studies



1 - 11

Scheme 1

Figure 1



2-isomer [I, II = R] 1-isomer [II, II = R]

(5,7-15) have clearly demonstrated that such disubstituted isomers can readily be differentiated with the help of proton magnetic resonance spectroscopy. The chemical shifts observed for the α-protons of the *N*-substituent have been shown to differ for two isomers (5 and 7-9). Such a difference in the chemical shift depends largely on the nature of the 5-substituent which can cause significant shielding or deshielding of the α-protons of the *N*-substituent, especially so in the case of 1,5-disubstitution. Steric factors also play an important role in determining the ratio of the two isomers formed. These results have shown that the 2-isomer is the predominant one (80-90%) obtained with 5-aryltetrazoles (10) while the ratio can approach unity with 5-alkyltetrazoles (11). Furthermore, an increase in the bulk of the *N*-substituent results in a marked decrease in the formation of 1-isomer. On the basis of these observations, substitution of 5-phenyltetrazole with chloroacetylcarbamides would be expected to result in the formation of at least two different products, namely 1,5- and 2,5-disubstituted tetrazoles with the predominance of the 2-isomer. Thus, one should also observe a difference in the chemical shift for the $-CH_2-$ protons of the 1- and 2-isomers.

Our present results have provided support for all of the above speculations. The recrystallization of the crude reaction product in all the cases yielded a sharp melting product that gave only one spot on tlc, was analytically pure, and in the nmr gave one peak for the $-CH_2-$ protons in the range of δ 5.75-5.90. However, the residue obtained after the evaporation of the mother liquor was found to be contaminated with another product on tlc. The nmr of the residue revealed the presence of two peaks for

$-CH_2-$ protons; one in the range of δ 5.75-5.90 and the other in the range of δ 5.55-5.65. Since the residue obtained in all the cases was of the order of about 5% of the total crude product, and since the peak height ratios for the two $-CH_2-$ proton peaks indicated the compound corresponding to the $-CH_2-$ peak occurring at lower ppm to be about 10-20% of the total residue, it was concluded that the latter was formed only to an extent of about 0.5-1.0%. A model study shows that the π-cloud of the 5-phenyl ring will exert a moderate shielding effect on the $-CH_2-$ group at N-1 of the tetrazole moiety. This is probably due to the large bulk of the substituent that does not allow the phenyl ring to be coplanar with tetrazole ring in case of substituent being present at N-1 rather than at N-2. We have therefore assigned structure I to the product with CH_2- protons occurring at higher ppm while the structure II has been assigned to the product with $-CH_2-$ protons occurring at lower ppm. This kind of reasoning is in good agreement with some of the earlier literature reports. No attempts were made to isolate the 1,5-disubstituted tetrazoles in the pure form.

All 2-(1-acetoxy-3-substituted carbamido)-5-phenyltetrazoles were tested at a dose of 100 mg./kg., i.p., for their ability to inhibit edema formation induced by carrageenin in rat paws. Compounds **6**, **7**, **10** and **11** were found to be inactive while the remaining compounds exhibited activity and the degree of protection against carrageenin-induced edema ranged from 11-41%. Compounds **3** and **5** were significantly active having 41% and 37% antiedema activity, respectively, as compared to oxyphenbutazone, used as a reference drug, which gave 51% protection when administered intraperitoneally at a dose of 40 mg./kg.

All compounds, with the exception of compounds **7**, **8**, **10** and **11**, showed significant inhibition of trypsin catalyzed hydrolysis of bovine serum albumin. All compounds were tested for their antiproteolytic activity at a final concentration of 1 m*M* and the degree of inhibition ranged from 9-66% for various compounds. Compounds **2**, **4** and **5** provided maximum inhibition of trypsin activity and the percent inhibition was 40, 66 and 35, respectively. Sodium salicylate, used as a standard reference drug, provided 55% inhibition at a final concentration of 1 m*M*.

Compounds **3**, **4** and **5** were further tested for their ability to provide protection against cotton pellet-induced granuloma formation in rats. All three compounds were found to be inactive as compared to oxyphenbutazone (100 mg./kg., i.p.) which provided 47% protection under similar experimental conditions.

These results have indicated low antiinflammatory activity of 2,5-disubstituted tetrazoles and hence no structure-activity-relationship of these could be demonstrated with respect to their antiinflammatory activity. The low antiinflammatory activity possessed by these compounds was found to exhibit no correlationship with their antiproteolytic activity.

EXPERIMENTAL

All compounds were analyzed for their carbon, hydrogen and nitrogen contents. Melting points were taken in an open capillary tube with an immersion thermometer and are corrected. The nuclear magnetic resonance spectra of compounds **1-11** were recorded on a Varian Associates EM-390 spectrometer in DMSO-d_6 using tetramethylsilane as an internal standard.

5-Phenyltetrazole was prepared by following the method of Finnegan, *et al.* (16). 1-Chloroacetyl-3-substituted carbamides were prepared by reacting equimolar quantities of appropriate carbamide and chloroacetylchloride in dry benzene. These substituted carbamides were characterized by their infrared spectra and by their melting points as reported earlier (17,18).

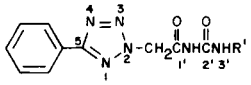
Table I

Physical Constants of 2-(1-Acetoxy-3-substituted Carbamido)-5-phenyltetrazoles

Compound No.	R	M.p. °C	Yield %	Molecular Formula	Analyses % Calculated C	H	N	Found C	H	N
1	CH_3	180	81	$C_{11}H_{12}N_6O_2$	50.77	4.61	32.30	50.71	4.62	32.36
2	C_2H_5	205	86	$C_{12}H_{14}N_6O_2$	52.55	5.11	30.66	52.52	5.12	30.66
3	*n*-C_3H_7	200	83	$C_{13}H_{16}N_6O_2$	54.17	5.55	29.17	54.26	5.50	29.41
4	*n*-C_4H_9	181	85	$C_{14}H_{18}N_6O_2$	55.63	5.96	27.82	55.41	5.90	28.11
5	C_6H_5	216	89	$C_{16}H_{14}N_6O_2$	59.62	4.34	26.08	60.12	4.38	26.26
6	*o*-$CH_3C_6H_4$	222 dec.	92	$C_{17}H_{16}N_6O_2$	60.71	4.76	25.00	60.70	4.73	25.01
7	*m*-$CH_3C_6H_4$	194	92	$C_{17}H_{16}N_6O_2$	60.71	4.76	25.00	60.76	4.71	25.21
8	*p*-$CH_3C_6H_4$	221	90	$C_{17}H_{16}N_6O_2$	60.71	4.76	25.00	60.81	4.71	25.40
9	*o*-$OCH_3C_6H_4$	238	93	$C_{17}H_{16}N_6O_3$	57.95	4.54	23.86	57.89	4.50	23.81
10	*p*-$OCH_3C_6H_4$	215	88	$C_{17}H_{16}N_6O_3$	57.95	4.54	23.86	57.90	4.52	23.90
11	*p*-$OC_2H_5C_6H_4$	220	87	$C_{18}H_{18}N_6O_3$	59.02	4.91	22.95	58.96	4.86	23.00

Table II

Nuclear Magnetic Resonance Spectral Data of 2-(1-Acetoxy-3-alkylcarbamido)-5-phenyltetrazoles (a,b)

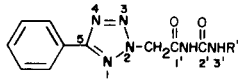


Compound No.	NH (1′)	NH (3′)	R	Aromatic Protons	-CH_2-(2-isomer)	-CH_2-(1-isomer)
1	10.75 (b)	7.80 (b)	2.70 (3H, d, J = 4 Hz)	7.6 (3H, m) 8.1 (2H, m)	5.75	5.55
2	10.75 (b)	7.89 (b)	1.05 (3H, t, J = 6 Hz) 3.30 (2H, q, J = 6 Hz)	7.5 (3H, m) 8.1 (2H, m)	5.75	5.55
3	10.75 (b)	7.85 (b)	0.76 (3H, t, J = 6 Hz) 1.43 (2H, q, J = 6 Hz) 3.06 (2H, q, J = 6 Hz)	7.5 (3H, m) 8.1 (2H, m)	5.75	5.55
4	10.75 (b)	7.85 (b)	0.70, 1.70 (7H, m) 3.15 (2H, m)	7.6 (3H, m) 8.1 (2H, m)	5.75	5.55

(a) Abbreviations: s = singlet; d = doublet; t = triplet; q = quartet; m = multiplet; b = broad. (b) Chemical shifts are reported in ppm.

Table III

Nuclear Magnetic Resonance Spectral Data of 2-(1-Acetoxy-3-arylcarbamido)-5-phenyltetrazoles (a,b)



Compound No.	NH (1′)	NH (3′)	R	Aromatic Protons	-CH_2-(2-isomer)	-CH_2-(1-isomer)
5	11.10 (s)	9.90 (s)	--	6.9-7.65 (8H, m) 7.9-8.3 (2H, m)	5.90	5.65
6	11.30 (b)	9.85 (s)	2.17 (3H, s)	6.9-8.3 (9H, m)	5.85	5.65
7	11.10 (b)	9.85 (s)	2.30 (3H, s)	6.9-8.3 (9H, m)	5.90	5.65
8	11.00 (b)	9.85 (s)	2.23 (3H, s)	6.9-8.2 (9H, m)	5.85	5.65
9	11.30 (b)	10.33 (b)	3.76 (3H, s)	6.85-8.25 (9H, m)	5.90	5.65
10	11.00 (b)	9.85 (s)	3.70 (3H, s)	6.7-8.2 (9H, m)	5.85	5.65
11	11.10 (b)	9.90 (s)	1.20 (3H, t, J = 8 Hz) 3.94 (2H, q, J = 6 Hz)	6.7-8.25 (9H, m)	5.90	5.65

(a) Abbreviations: s = singlet; d = doublet; t = triplet; q = quartet; m = multiplet; b = broad. (b) Chemical shifts are reported in ppm.

2-(1-Acetoxy-3-substituted Carbamido)-3-phenyltetrazoles.

A mixture containing 5-phenyltetrazole (0.02 mole), appropriate 1-chloroacetyl-3-substituted carbamide (0.02 mole) and triethylamine (0.02 mole) in dry acetone was refluxed on a steam bath for 8 hours. The reaction mixture was filtered while hot and the solvent evaporated under reduced pressure. The residue was washed several times with hot water, collected by filtration and dried. Recrystallization from acetone gave the desired product. However, the mother liquor was evaporated to dryness and the residue was saved for the identification of the isomeric 1-(1-acetoxy-3-substituted carbamido)-5-phenyltetrazoles.

The antiedema activity of these compounds was determined in rats by following the method reported earlier (19). The compounds were administered intraperitoneally at a dose of 100 mg./kg. The edema was produced by injecting 0.05 ml. of a 1% suspension of carrageenin in normal saline in the aplantar aponeurosis of the right hind paw. The antigranuloma activity was also determined in rats at a dose of 100 mg./kg. (i.p.) by following the method reported earlier (19).

The ability of these compounds to inhibit trypsin catalyzed hydrolysis of bovine serum albumin was tested for their antiproteolytic activity at a final concentration of 1 m*M* using DMSO as the solvent (19).

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