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3-DIAZOQUINOLINE TETRAFLUOROBORATE AS A REAGENT FOR SPECIFIC MODIFICATION OF LYSYL RESIDUES: UNIQUE STRUCTURE OF THE REACTION PRODUCT*

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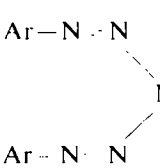
SUMMARY

3-Diazoquinoline tetrafluoroborate can modify some of lysyl residues of proteins, such as trypsin, lysozyme and insulin, at pH 8.2 and 4 °C. Tyrosyl and histidyl residues are not reactive to this diazonium salt. The modified lysyl residue was revealed to have a structure of 1-substituted 1,2,3-triazolo[4,5-*c*]quinoline, quite different from those of the usual azo-coupling products.

It was previously reported that 3-diazoquinoline tetrafluoroborate showed unusual effects on both bovine pancreatic trypsin [1] and chymotrypsin [2]. The reagent modified trypsin exclusively at its 3 out of 14 lysyl residues, accompanying enhancement of the activity toward some synthetic substrates, contrary to the expectation that the azo-coupling would occur preferentially at tyrosyl and histidyl residues [1].

The possibility that ε-amino groups of lysyl residues of proteins react with aromatic diazonium compounds in one to two stoichiometry yielding pentazene derivatives [3] (I) has been pointed out by several reports [4–7]. In our previous study [1], however, the reaction products of ε-amino-*n*-caproic acid and N^{α}-benzyloxycarbonyl-(Z-)-L-lysine with 3-diazoquinoline tetrafluoroborate were shown to have the elemental compositions which agreed well with those for triazene (1,3-disubstituted) derivatives [8] (II).



(I)

(II)

Abbreviations: Z-, benzyloxycarbonyl-.

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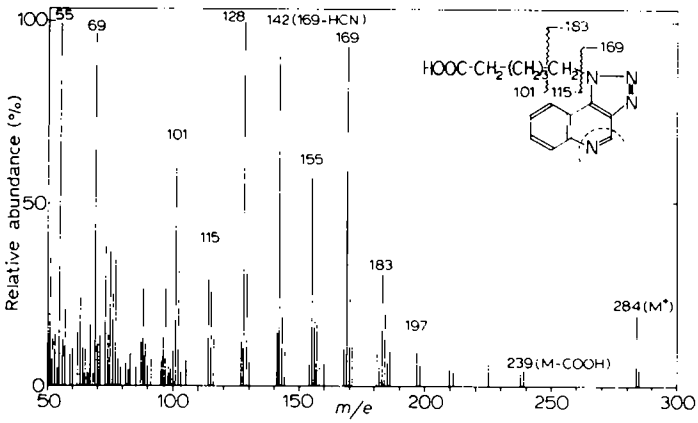
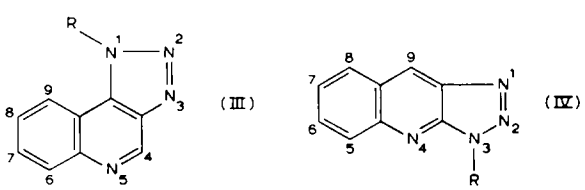


Fig. 1. Mass spectrum of 1-triazolo[4,5-*c*]quinolinehexanoic acid.

Therefore, they were tentatively identified as N^{ε}-(quinoline-3-yl)-azo amino derivatives. Nevertheless, remarkable stability of the products against various treatments including acid and alkaline hydrolyses was hardly explainable by triazene structure [9]. It seemed necessary to establish molecular structures of the 3-diazoquinoline-modified amino compounds in order to investigate the specific feature of the reaction of the reagent with trypsin leading to the enhancement of activity.

Mass spectrometric analysis of the compound obtained by the reaction of 3-diazoquinoline tetrafluoroborate with ε-amino-*n*-caproic acid was then carried out with a Hitachi Model RMU-6E mass spectrometer. The spectrum is shown in Fig. 1. A parent peak was observed at *m/e* 284 which was smaller than the molecular weight of the triazene (1,3-disubstituted) derivative by two mass units. The fact suggested an intra-molecular ring structure and the following alternative triazole derivatives were taken into consideration, namely, 1,2,3-triazolo[4,5-*c*]quinoline (III) and 1,2,3-triazolo[4,5-*b*]quinoline (IV).



High abundance of a fragment peak of *m/e* 142 (possibly 169–HCN), however, strongly supported the former structure, because the fragment could hardly be expected from the latter structure abundantly. The existence of the base peak of *m/e* 128 (possibly 142−N) may also be in support of III.

Comparison of the characteristics in ultraviolet absorption of the compound with those of several triazoloquinolines reported by Fleet and Fleming [10] evidenced that 1-triazolo-[4,5-*c*]quinolinehexanoic acid was the product (see Table I). (Found: C, 63.27%; H, 5.75%; N, 19.88%; molecular weight determined by high-resolution

TABLE I

CHARACTERISTICS IN ULTRAVIOLET ABSORPTION OF TRIAZOLOQUINOLINES

Wavelengths of maximal absorption (λ_{max}) and molar absorptivities (ε) in ethanol.

Compound							
1-Triazolo[4,5-*c*]quinoline-hexanoic acid*	λ_{max} (nm)		237	283	306	320	
	ε ($M^{-1} \cdot cm^{-1}$)		39 400	4740	2200	2100	
Triazolo[4,5-*c*]quinoline**	λ_{max} (nm)	217	239	281	306	320	
	ε ($M^{-1} \cdot cm^{-1}$)	27 100	33 400	5540	2500	2160	
Triazolo[4,5-*b*]quinoline**	λ_{max} (nm)		237			322	351
	ε ($M^{-1} \cdot cm^{-1}$)		35 900			9980	4680

* Present study.
** By Fleet and Fleming [10].

mass spectrometry, 284.1267. $C_{15}H_{16}O_2N_4$ requires: C, 63.36%; H, 5.67%; N, 19.71%; mol. wt 284.1273).

NMR spectrometry performed with a Hitachi Model H-60 (60 MHz) gave further confirmation (Fig. 2). A sharp singlet peak of δ = 9.48 in dimethyl sulfoxide-d_6 at room temperature is marked, which corresponds to a proton at 4-position of the triazolo[4,5-*c*]quinoline nucleus. A simpler compound was prepared from β-alanine by the same method as from ε-amino-*n*-caproic acid and its structure was also

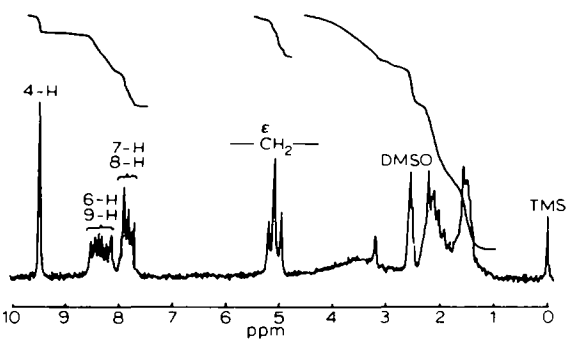


Fig. 2. NMR spectrum of 1-triazolo[4,5-*c*]quinolinehexanoic acid.

examined. As shown in Fig. 3, the NMR spectrum of the product was essentially identical with that of the hexanoic acid derivative and all peaks were assigned successfully. (1-Triazolo[4,5-*c*]quinolinepropionic acid, m.p. 239–244 °C (decompn), found: C, 59.58%; H, 4.28%; N, 23.39%. $C_{12}H_{10}N_4O_2$ requires C, 59.50%; H, 4.16%; N, 23.13%) Reaction of the reagent with ethylamine gave a compound which was almost identical to III in the spectral behavior, too. All the properties previously reported [1] on the reaction product of 3-diazoquinoline tetrafluoroborate and N^{α}-Z-L-lysine are compatible with the presence of the same ring structure also in the product.

Thus, the formation of 1-substituted 1,2,3-triazolo[4,5-*c*]quinoline may be generally encountered in the reaction of 3-diazoquinoline tetrafluoroborate with *o*-amino compounds. In any of the other aromatic diazonium reagents tested, however,

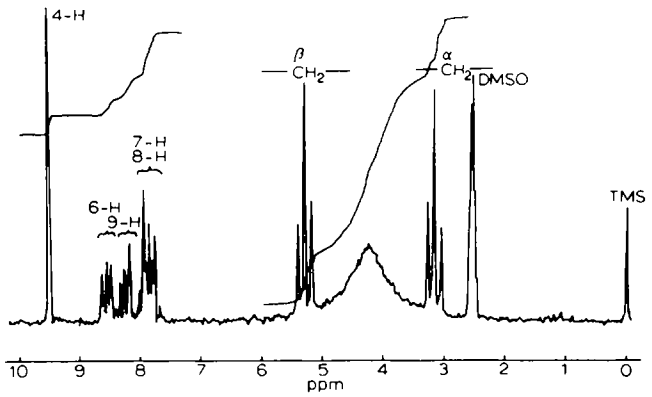


Fig. 3. NMR spectrum of 1-triazolo[4,5-*c*]quinolinepropionic acid.

1,2,3-triazole derivatives have not yet been found to occur. As described in the previous paper [1], 3-diazoquinoline-modified derivatives of bovine pancreatic trypsin and of N^{α}-Z-L-lysine showed quite similar spectral properties and their acid hydrolyzates gave an identical new peak on the chart of an amino acid analyzer (the short column). It may be concluded therefore that the specific reaction of 3-diazoquinoline tetrafluoroborate at lysyl residues introduced this unique ring structure also in the trypsin molecule.

Hen egg-white lysozyme and bovine pancreatic insulin were allowed to react with 10-fold molar excess of the reagent in 50 mM Tris–HCl buffer (pH 8.2) for 30 min at 4 °C. By the analysis of amino acid compositions and the spectrophotometric observation, the reaction was proved to occur also exclusively at lysyl residues yielding the products of the same type as in trypsin. The preferential modification of only 2 out of 6 residues of lysine was observed in the case of lysozyme even by the use of 20-fold molar excess of the reagent. The similar situation has been experienced with trypsin [1]. The prompt modification of lysyl residues with 3-diazoquinoline tetrafluoroborate seems to require the intact conformation of proteins, because the addition of 8 M urea in the medium resulted in remarkable reduction of the reaction rate without affording any noticeable influence on the reactivity of the reagent toward simple ω-amino compounds. Fluorescence was observed when the triazoloquinolines were excited at around 321 nm. The emission spectra of 3-diazoquinoline-modified trypsin and lysozyme were somewhat different from those of the model compounds in the same solvent, probably reflecting the respective environmental characteristics of the modified residues.

REFERENCES

1 Kanazawa, M., Yoshida, N. and Ishii, S. (1971) Biochim. Biophys. Acta 250, 372–389
2 Yoshida, N. and Ishii, S. (1972) J. Biochem. Tokyo 71, 185–191
3 Suling, C. (1965) in Methoden der Organischen Chemie (Muller, E., ed.), Vol. X/3, p. 736, Georg Thieme Verlag, Stuttgart, Vierte Auflage
4 Howard, A. N. and Wild, F. (1957) Biochem. J. 65, 651–659
5 Higgins, H. G. and Harrington, K. J. (1959) Arch. Biochem. Biophys. 85, 409–425

6 Tabachnick, M. and Sobotka, H. (1959) J. Biol. Chem. 234, 1726–1730
7 Phillips, Jr, J. H., Robrish, S. A. and Bates, C. (1965) J. Biol. Chem. 240, 699–704
8 Suling, C. (1965) in Methoden der Organischen Chemie (Muller, E., ed.), Vol. X/3, p. 695, Georg Thieme Verlag, Stuttgart, Vierte Auflage
9 Kawanishi, K., Otani, I. and Nozaki, H. (1968) Tetrahedron Lett. 53, 5575-5576
10 Fleet, G. S. J. and Fleming, I. (1969) J. Chem. Soc. (c) 1758-1763