NOTES

An Iodometric Assay of Some Derivatives of 7-Aminocephalosporanic Acid

By Sachiko OKADA, Kiyoshi HATTORI and Tadayoshi TAKANO

(Received February 20, 1965)

The hydroxylamine method[1] and the iodometric assay are widely used for the determination of the purity of penicillins. Cephalosporin-C (II) is an antibiotic with a structure similar to penicillin; Alicino has reported an iodometric assay of this substances.[2]

An iodometric assay for some derivatives of 7-aminocephalosporanic acid (I) has now been studied. As the β-lactum ring of these derivatives is generally more stable than that of penicillins upon acidic and basic hydrolyses, only the conditions of the basic hydrolysis have been changed in the iodometric assay.

In Fig. 1, the time of hydrolysis is shown against the consumption of iodine. The experimental method has been standardized as follows: a sample

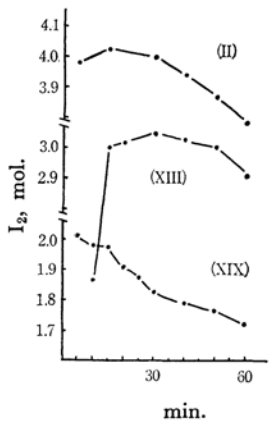
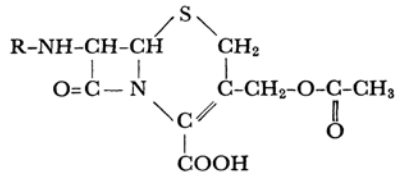


Fig. 1. The relation between I_2 consumption and time of basic hydrolysis; the number of derivative indicated in Table I.

1) J. H. Ford, *Ind. Eng. Chem., Anal. Ed.*, **19**, 1004 (1947).
2) J. F. Alicino, *Anal. Chem.*, **33**, 648 (1961).

(3—5 mg.) is dissolved in 5 ml. of a phosphate buffer (pH 6.0). To this solution is then added the same volume of N sodium hydroxide, and the mixture is kept at 23—25°C for fifteen minutes. Five milliliters of 1.2 N hydrochloric acid and 10 ml. of a 0.01N iodine solution are then added in order. Fifteen minutes later the residual iodine is titrated with 0.01 N sodium thiosulfate.

TABLE I. IODINE COMSUMPTION OF 7-AMINOCEPHALOSPORANIC ACID DERIVATIVES



No. (derivatives)	R- (substituents)	Iodine consumption mol.
I	Hydrogen	2.90
II	δ-Aminoadipoyl	4.01
III	10-Undecenoyl	3.62
IV	α-Chlorobutyroyl	3.59
V	Phenylacetyl	4.18
VI	*p*-Tolylacetyl	3.98
VII	*p*-Anisylacetyl	4.76
VIII	α-Thienylacetyl	3.95
IX	*p*-Chlorophenoxyacetyl	4.15
X	*o*-Nitrophenoxyacetyl	3.68
XI	Cinnamoyl	3.88
XII	β-(5-Nitro-2-furyl)acryloyl	4.10
XIII	α, β-Dichloro-β-phenylpropionoyl	3.10
XIV	α-Phenyl-β-(*p*-anisyl)propionoyl	4.54
XV	*N*-Benzyloxycarbonyl-*C*-phenylglycyl	4.63
XVI	Ethoxycarbonyl	4.01
XVII	Phenoxycarbonyl	4.63
XVIII	Phenylcarbamoyl	3.86
XIX	α-Naphthylcarbamoyl	1.97

The results are listed in Table I.

The *N*-acylation of the 7-amino group gives evidence of an iodine consumption differing from that of the original 7-aminocephalosporanic acid (I); this behavior is similar to that between penicillin and 6-aminopenicillanic acid. Under these conditions, iodine consumption is not observed for the substituent parts—i. e., cinnamic acid, β-(5-nitro-1-furyl)acrylic acid, phenylacetic acid, *p*-chlorophenoxyacetic acid and 10-undecenoic acid.

Even when the substituents are sturcturally classified, there is no regular relationship between the iodine consumption and any of the groups. However, the reproducibility of the iodine consumption in this assay is high with respect to each of the derivatives. Hence, this assay might be useful for the inspection of the purity of each compound. The decreasing curves of iodine consumption in Fig.1 indicate that some destruction of the active structure or some formation of a reductive substance might occur during a long basic hydrolysis. The nature of the active structure in this assay remains to be studied, however.

Research Laboratories
Fujisawa Pharm. Co., Ltd.
Higashiyodogawa, Osaka