detected by the X-ray-diffraction technique whereas a similar phenomenon in solution is seen by optical-rotation methods. The disappearance of the original α-pattern might be explained in terms of small distortions not requiring wholesale breakdown of the secondary structure. It is not clear how supercontraction and elastomeric behaviour would be explained on this basis. (b) In solid protein the reaction due to LiBr may be very different from the reaction in solution. LiBr is absorbed in substantial quantity by the solid and its penetration alone causes a physical disturbance which must involve some forcing apart of molecular chains. We thus have physical and chemical effects to consider and both inter-chain and intra-chain bonds might be weakened or broken. In solution the question of penetration in the same sense does not arise and H-bonds may increase in stability.

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The application of the Sakaguchi reaction to the determination of DNP-arginine

During the study of an N-terminal amino acid residue of a protein by the dinitrophenylation procedure[1] it is necessary, when the end-group is arginine, to be able to determine accurately the amount of DNP-arginine present in the mixture of amino acids resulting from the acid hydrolysis of the dinitrophenylated protein. A method whereby this may be achieved has been outlined by WISSMANN AND NITSCHMANN[2]. In this, the DNP-arginine is first separated from the accompanying free amino acids by chromatography on talc columns, and subsequently subjected to a modification[3] of the Sakaguchi reaction, the colour produced being compared with that from similarly treated standards. The validity of the determination is therefore dependent on the assumption that DNP-arginine behaves similarly to arginine when treated with alkaline hypobromite.

When attempts were made to determine the DNP-arginine released by hydrolysis of dinitrophenylated α-casein using this method, very variable results were obtained. It was found that in order to obtain reproducible results with DNP-arginine, the rate

Abbreviation: DNP-, 2,4-dinitrophenyl-.

of addition of the reactants, and the time allowed for colour development required more strict control than was apparently necessary for the accurate determination of arginine itself. This stricter control has been incorporated in MACPHERSON's modification[3] of the Sakaguchi reaction, as follows.

Portions of a solution of DNP-arginine in 0.1 *N* HCl, usually about 3 ml and containing 50–500 μg DNP-arginine, are neutralised by addition of 10% KOH, and the addition continued until an excess of 1 ml has been added. This is followed by 2 ml of 0.1% α-naphthol in 50% ethanol, and 1 ml 40% aq. urea. Water is then added until the final volume of each mixture is 14 ml. To each, 1 ml of a solution containing 2 g bromine in 100 ml 5% KOH is now added in a dropwise manner and with shaking, over exactly 25 sec. After a further 2 min, 1 ml of 40% urea is added followed by a second addition of 1 ml of the hypobromite solution, again over 25 sec. The reaction mixture is allowed to stand at room temperature for 10 min and is then diluted to 25 ml with water. After a further 10 min the light absorption of the solution at 525 mμ is measured against a blank determination containing no DNP-arginine. The colour produced increased linearly with weight of DNP-arginine up to at least 1500 μg, and was sufficiently intense for reproducible results to be obtained from as little as 70 μg.

However, DNP-amino acids especially in solution are subject to photolytic decomposition[4–7], and reliable results were obtained with the above method only when the DNP-arginine was of recent preparation, and the solutions employed were shielded from light. In an attempt to eliminate the necessity for these precautions, and to provide a stable and more easily purified standard material, the determination was performed with L-arginine. It was found that while the results were again reproducible, the amount of colour produced by pure DNP-arginine was in excess of that produced by a molecular equivalent of L-arginine by between 40 and 100% depending upon the exact conditions used. Furthermore, the wavelength of maximum light absorption found for the DNP-arginine colour was approximately 525 mμ whereas that of the L-arginine colour was 505 mμ, as shown in Fig. 1. It might be supposed that this enhanced value for DNP-arginine is due directly to the presence of the dinitrophenyl chromophore whose wavelength of maximum light absorption is approximately 355 mμ. That this is not so can be seen from Fig. 1 where DNP-arginine in alkaline solution is shown to exhibit no selective light absorption in the region of 525 mμ. It is also apparent that although DNP-arginine obtained by hydrolysis of a dinitrophenylated protein is not separated by chromatography on talc from the accompanying ε-DNP-lysine, 2,4-dinitrophenol, and 2,4-dinitroaniline, the DNP groups of these substances having no absorption at 525 mμ cause no interference in the determination.

It was concluded that the difference in colour production between arginine and DNP-arginine, which makes the use of L-arginine as a standard undesirable, is due to differences in reactivity of the guanido groups in the two molecules brought about by the presence in one of them of the DNP group, at a point remote from the site of the primary reaction. To check this, two further substituted guanidines, methyl guanidine ($CH_3NHC[=NH]NH_2$), and guanidoacetic acid ($HOOCCH_2NHC[=NH]NH_2$), were treated with hypobromite under the same conditions used for DNP-arginine and L-arginine. It was found that while the density of the colour produced by L-arginine was almost identical with that from a molecular equivalent of guanido-

acetic acid it was again less than that produced by DNP-arginine, and very much less than that from methyl guanidine. This is illustrated in Fig. 2. These findings can be explained by supposing that there is a weak interaction between the acidic and basic extremities of the arginine and guanidoacetic acid molecules which prevents full colour development in the Sakaguchi reaction. No such interaction is possible with methylguanidine, while in DNP-arginine the presence of a substituent as large as the dinitrophenyl group in close proximity to the carboxyl group might be expected to prevent or at least hinder such an interaction. This being so, the guanido groups of both substances would be free to react more easily with alkaline hypobromite than the corresponding grouping in either L-arginine or guanidoacetic acid.

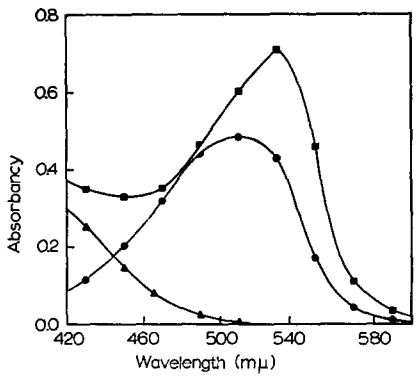

Fig. 1. ▲, absorption spectra of DNP-arginine in alkaline solution; ●, products resulting from Sakaguchi reaction on arginine and ■, DNP-arginine. In each the concentration of arginine derivative was 1.10 μmoles/25 ml.

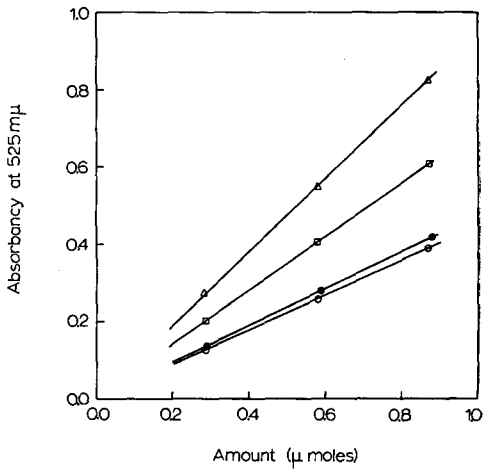

Fig. 2. Colour produced by Sakaguchi reaction from; ○, arginine; ●, guanidoacetic acid; □, DNP-arginine and △, methyl guanidine.

In summary, application of the Sakaguchi reaction to the determination of DNP-arginine has yielded results of sufficient accuracy to be valuable in the study of protein end-groups, only when the precautions and modifications outlined above have been observed, and in particular when the standards employed were prepared from DNP-arginine of the highest purity, *i.e.* having m.p. 258–259° and ε_{max} = 17,600 at 355 mμ in 1 % NaOH.

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