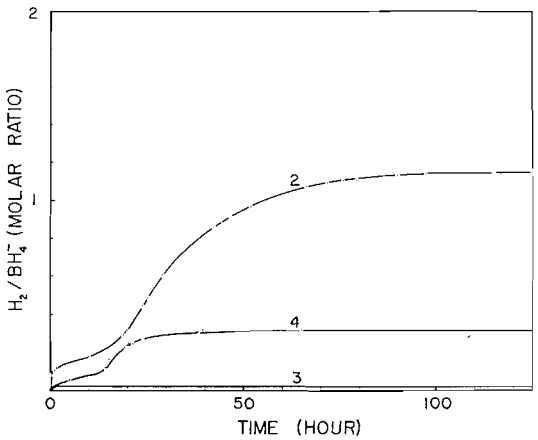

FIG. 4. Corrected volume of hydrogen evolved from a mixture of BH_4^- (0.10 mole) and Te (0.30 mole) in the presence of diglyme, at 56°. Reactions with $NaBH_4$(*2*), KBH_4(*3*), and $(CH_3)_4BH_4$(*4*).

Tellurium

As with selenium, tellurium gave a very fast and even explosive reaction with lithium borohydride in the presence of diglyme. With sodium borohydride, the reaction was very slow but stopped when two hydrogens of the borohydride were replaced by tellurium. Tetramethylammonium borohydride reacted only slightly and potassium borohydride gave no evolution of hydrogen.

These experiments indicate that of the possible combinations of hydrides and chalcogens, only the reaction of sodium borohydride with selenium and tellurium led to relatively stable compounds having the stoichiometry $NaBH_2M_3$ (M = Se, Te). In all other cases, the reaction was either too fast, too slow, or did not stop at a definite level in the course of replacement of the hydrogens by the chalcogens. In the case of $NaBH_2Se_3$, although the hydrogen evolution and subsequent hydrolysis indicated the above formulation, it has not been possible to remove this compound from its solvent, the reddish, rubber like substance retaining important portions of diglyme, even after repeated washings with petroleum ether. This difficulty prevented the elementary analysis as obtained for $NaBH_2Te_3$. The sodium borohydride incorporating tellurium was easily obtained as a black powder, free from diglyme, and showing relative stability for some hours. It is hygroscopic and sensitive to light.

The sodium borohydride incorporating tellurium still retains two hydrogens showing reducing capacity towards organic substrates. Since the anion $BH_2Te_3^-$ is even bulkier than $BH_2S_3^-$, the stereospecificity already observed with the sulfurated borohydride should be enhanced with $BH_2Te_3^-$.

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Electrolysis in liquid ammonia solution in peptide chemistry

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An adaptation of an electrolytic method is suggested for the removal of protecting groups in peptide chemistry. Some initial experiments are reported indicating the scope and possible limitations of the method.

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Introduction

The benzyl group ($C_6H_5CH_2$—) has had a wide use in peptide chemistry, since its introduction by du Vigneaud *et al.* (1) as a protective function. When it is used to protect the thiol group of cysteine the normal method of removal has been, until recently, reduction by sodium and liquid ammonia (Na/NH_3) (2). In 1965, Sakakibara and Shimonishi (3) found that the benzyl group in *S*-benzyl cysteine peptides could be

removed by the use of anhydrous HF. This reagent also removes other groups such as the *N*-carbobenzoxy, *t*-butyloxycarbonyl, benzyl esters, and benzyl ethers used as protective groups in peptide synthesis (4, 5). Unfortunately anhydrous HF does not remove the benzyl group from im.-benzyl histidine, and where benzyl protected cysteine and histidine occur in the same peptide, Na/NH_3 reduction still has to be used.

Du Vigneaud *et al.* (6) showed that in the removal of *S*-benzyl groups by Na/NH_3 reduction of a protected nonapeptide, high yields of physiologically active oxytocin could be obtained. However, Zahn *et al.* (7) and Katsoyannis (8) have indicated that when Na/NH_3 reduction is used on larger peptides, such as the A and B chains of insulin, low yields of the required products are obtained. To avoid the use of Na/NH_3 reduction many other protecting groups for —SH have been suggested in the literature (4, 5), but the benzyl group still seems the most popular.

The blue color obtained on dissolving alkali metals in liquid ammonia or low molecular weight amines has been suggested (9) as being due to the solvated electron. This species is also credited with being the reducing agent in these solutions. The color can also be obtained by electrochemical methods using these solvents (10). Electrolytic reduction of aromatic hydrocarbons in methylamine solution in the presence of LiCl was observed by Benkeser (10) and a similar reduction of estrone was observed by Fentiman and Poirier (11).

Since it was felt that this alternate method of electrolytically generating the active species in ammonia might find use in the field of peptide chemistry, several experiments were carried out to investigate the usefulness of this method for the removal of the benzyl group usually used to block cysteine peptides. Experimental conditions similar to those in the Na/NH_3 reduction procedure were employed in order that comparison of the results from experiments where both procedures were used could be made.

Experimental

The reductions were conducted in a 250 ml three-necked flask, the two side arms being fitted with coiled platinum wire electrodes and the centre neck being fitted with a soda lime drying tube. Liquid ammonia was collected in the flask by condensation of the gas from a tank, one of the electrodes being removed to allow for the inlet. All the materials used dissolved readily in the ammonia and during the electrolysis the solutions were stirred using a teflon coated magnetic stirring bar. The solutions were kept at acetone/dry ice temperature throughout the period of electrolysis, which was normally 90 min. The reduction could be followed by the decrease in resistance of the solution as evidenced by the increase in current flow at constant voltage. This decrease was complete within the 90 min period.

The materials used were synthesized by established methods and the constants agreed with published figures. The protected nonapeptide was synthesized by the stepwise active ester method (12) and the tetrapeptide by a combination of mixed anhydride and active ester techniques (12). The products of the reactions were usually analyzed by hydrolysis followed by chromatography using a Beckman-Spinco Amino acid analyzer, Model 120C. It was established that under the hydrolysis conditions used there were only trace amounts of cysteine produced from the *S*-benzyl cysteine.

The power source was a Heathkit constant voltage supply unit with a maximum of 400 V D.C. at 100 mA.

Electrolytic Reduction of a Protected Nonapeptide to Oxytocin

The protected nonapeptide Z-*S*-bzl-cys-tyr-ileu-gln-asn-*S*-bzl-cys-pro-leu-gly NH_2 (200 mg) was dissolved in liquid ammonia (125 ml) and electrolyzed for 90 min. The initial current was 20 mA at 400 V D.C. After the 90 min period the current had risen to 75 mA at the same voltage and was constant. The ammonia was allowed to evaporate and the last 10 ml removed under vacuum. The resulting solid was dissolved in water (50 ml) and the pH adjusted to 7.0 with dilute acetic acid. A sample (5 ml) was withdrawn, the solvent removed under vacuum and the product hydrolyzed in 6 *N* HCl (1 ml) for 16 h at 110 °C under vacuum. Analysis of the solution showed cysteine present in the correct ratio, no *S*-benzyl cysteine was observed.

Air was bubbled through the remaining solution (45 ml) for 2 1/2 h and, after the pH had been adjusted to 4.0 with dilute acetic acid, it was filtered. Assay of the solution using the isolated rat uterus method gave 390 IU/ml of oxytocic activity or an adjusted total of 21 400 International oxytocic units from 200 mg of protected nonapeptide.

When the experiment was repeated with the addition of NH_4Cl (1 g) to the liquid ammonia solution the observed current was 100 mA at 40 V D.C. This remained constant for 90 min. No oxytocic activity was found in the product and hydrolysis in 6 *N* HCl and analysis showed that all the cysteine was present as the *S*-benzyl derivative.

In a comparable experiment where the reduction was performed using Na/NH_3 instead of electrolysis, the yield was 20 500 oxytocic units from 200 mg of protected nonapeptide. There was no *S*-benzyl cysteine in an hydrolysate of the product.

Electrolytic Reduction of Protected Tetrapeptide

The protected tetrapeptide, Z-*S*-bzl-cys-pro-leu-gly NH_2 (200 mg) was subjected to the electrolytic reduction detailed above. The current was initially 20 mA at 400 V and rose to 90 mA during the 90 min reaction period. A small amount of the product, which would not

crystallize, was hydrolyzed in 6 *N* HCl and analyzed for amino acid composition. Both cysteine and *S*-benzyl cysteine were present in the ratio of about 7:3. Repetition of the experiment gave the same result. The product of the reaction also gave a positive ninhydrin reaction indicating the removal of the *N*-carbobenzoxy group.

Attempted Reduction of Z-S-Bzl-cysteine

No reduction was observed when Z-*S*-bzl-cysteine was used in the described method. The product obtained after removal of the ammonia could be crystallized from ethyl acetate/petroleum ether and was shown by m.p., mixed m.p., and rotation to be starting material. Hydrolysis and analysis showed only the presence of *S*-benzyl cysteine. The current started at 100 mA at 100 V D.C. and stayed at this level throughout the 90 min period of reaction. Na/NH_3 reduction and hydrolysis of the product showed no *S*-benzyl cysteine.

Attempted Reduction of Z-S-Bzl-cysteine Methyl Ester

When Z-*S*-bzl-cysteine methyl ester was used in the electrolytic method and the product hydrolyzed after removal of the ammonia, all the cysteine was found as *S*-benzyl cysteine. The current started at 20 mA at 400 V and dropped slowly to 10 mA at the end of 90 min. Again Na/NH_3 reduction and hydrolysis gave a product with no *S*-benzyl cysteine.

Discussion

It is apparent from the experimental results, that, with the conditions used, there is electrolytic reduction taking place.

When the protected nonapeptide was used, both the *S*-benzyl and *N*-carbobenzoxy groups were removed, since the product had the same biological activity as that obtained from a Na/NH_3 reduction of the same starting material. With the protected tetrapeptide only partial reduction occurred as evidenced by the presence of some *S*-benzyl cysteine in the hydrolysate, whereas no reduction was found with any of the cysteine derivatives used. The mechanism of the reduction would appear to be different from the Na/NH_3 reduction, since the simpler derivatives of cysteine are untouched by the electrolytic method, but are completely reduced by Na/NH_3. When a protected nonapeptide, obtained by the solid phase synthetic method, was substituted, on one occasion, for the regular nonapeptide, no reduction was observed. The peptide from this method of synthesis differed in having *O*-benzyl tyrosine rather than the free tyrosine as in the regular nonapeptide. This would imply that a partially ionizable group was necessary for reduction to occur. At no time was any blue color observed in the reaction vessel to indicate the presence of solvated electrons, although this does not preclude the reduction proceeding through a very short lived species. The particular electrolytic reduction observed in the above experiments would also appear to have a different mechanism from that observed by Benkeser *et al.* (10), since an introduction of a species designed to increase current flow (NH_4Cl) actually inhibited the reduction.

Future work is planned to widen the scope of the reaction, i.e. the removal of other protecting groups used in peptide chemistry, such as *O*-benzyl, *N*-tosyl, and the nitro group used in arginine. Further information is also required to determine the mechanism of the reaction and whether trace amounts of ions are required.

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