Improved Synthesis of Bradykinin

One of the major problems of peptide synthesis is protection of the guanido function of arginine. This has been accomplished by a variety of methods including even protonation (cf. SCHRÖDER and LÜBKE[1]). There has been no really adequate solution to the simultaneous requirements of introduction of the protecting group in high yield, inertness to the usual reagents and conditions of peptide synthesis, and removal in high yield under mild conditions.

Probably the most attractive guanido protecting group would be *p*-toluene-sulfonyl were it not that sodium-liquid ammonia reduction is required for its removal. We have now found that liquid hydrogen fluoride at 0° C removes the N^G-tosyl group rapidly and cleanly[2]. The synthesis of bradykinin provides an example.

Z-Arg(Tos)-Pro-Pro-Gly-Phe-Ser-Pro-Phe-Arg(Tos)[3,4] was hydrogenated[6] in 90% acetic acid over palladium black at 4 atmospheres and room temperature. The crude product was purified by countercurrent distribution[7] in methanol-water-chloroform-carbon tetrachloride 37:10:26:27 for 200 transfers. Arg(Tos)[1]-Arg(Tos)[9]-bradykinin was obtained as an amorphous powder, giving a theoretical curve in the above countercurrent distribution, K = 2.33, and homogeneous on silica t.l.c. in *n*-butanol-acetic acid-water 7:1:2, Rf. 0.41. $[\alpha]_D^{24} - 56°$, $c = 1$, acetic acid. Anal.[9] Calcd. for $C_{64}H_{85}N_{15}O_{15}S_2 \cdot 3H_2O$: C, 54.03; H, 6.45; N, 14.77; S, 4.51. Found: C, 54.26; H, 6.58; N, 14.59; S, 4.53. Amino acid analysis[10]: Arg(Tos) 2.0; Pro 3.0; Gly 1.0; Phe 2.1; Ser 0.9. Duplicate bioassays[11] gave potencies of 2.3×10^{-5} and 4.1×10^{-5} bradykinin. Surprisingly, the dose-response curves for standard and test compound were parallel. No inhibition was observed.

A sample of Arg(Tos)[1]-Arg(Tos)[9]-bradykinin (0.500 g) was dissolved in 25 ml of liquid hydrogen fluoride and the solution stirred $^1/_2$ h at 0° C. The solution was placed in a 20° C bath and the hydrogen fluoride pumped off under vacuum over a period of about $^1/_2$ h[12]. The residue was dried in a vacuum desiccator over potassium hydroxide pellets and purified by gradient elution chromatography[7] on a weakly acidic ion exchange resin (IRC-50) using a linear gradient from 0.1 *N* acetic acid to glacial acetic acid.

The appropriate fractions were combined and lyophilized giving bradykinin as a white powder, 0.298 g (68% yield calculated as the triacetate). Amino acid analysis: Arg 2.1; Pro 2.8; Gly 1.0; Phe 2.1; Ser 0.9. Duplicate bioassays gave potencies of 1.35 and 1.25 bradykinin. We may have the purest bradykinin yet reported. The hydrogen fluoride method is now being used in the synthesis of potential competitive inhibitors of bradykinin.

Zusammenfassung. Eine neue Methode zur Entfernung der Schutzgruppe von N^G-Tos-Arg mittels wasserfreier Flußsäure und ihre Anwendung für die Synthese von Bradykinin wird beschrieben.

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[2] S. SAKAKIBARA, Y. SHIMONISHI, Y. KISHIDA, M. OKADA and H. SUGIHARA, Bull. chem. Soc. Japan *40*, 2164 (1967). These authors reported the tosyl group to be stable in liquid HF at 20°.
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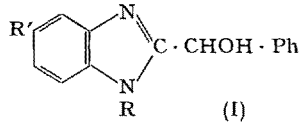
[4] All amino acids have the L-configuration. The following abbreviations are used[5]: Z, carbobenzyloxy; Tos, *p*-toluenesulfonyl.
[5] IUPAC-IUB Commission on Biochemical Nomenclature, Biochemistry 5, 2485 (1966).
[6] Hydrogenation: W. M. SELBY.
[7] Countercurrent distribution and ion-exchange chromatography: R. DAHM. All purifications were followed by silica t.l.c. and spots detected by the *t*-butyl hypochlorite-starch-iodide method[8].
[8] R. H. MAZUR, B. W. ELLIS and P. S. CAMMARATA, J. biol. Chem. *237*, 1619 (1962).
[9] Elemental analyses: E. ZIELINSKI.
[10] Amino acid analyses: J. W. AHLBERG.
[11] Bioassays: J. H. SANNER. The effect on guinea-pig ileum was compared with standard bradykinin (Sandoz Pharmaceuticals, Hanover, New Jersey, BRS-640, Lot 64003).
[12] Under these conditions N^G-Tos-Arg was quantitatively converted to Arg while N^ϵ-Tos-Lys and Tos-Leu were each deprotected to the extent of about 0.1% as determined by t.l.c. comparison with solutions of known concentration.

D-5-Chloro-2-(α-hydroxybenzyl)benzimidazole and 1-Alkyl-5-chloro-2-(α-hydroxybenzyl)benzimidazoles as Inhibitors of Poliovirus Multiplication

As 5-chloro-2-(α-hydroxybenzyl)benzimidazole (5-chloro-HBB) (I; R = H, R′ = Cl) markedly inhibits the multiplication of type 2 poliovirus[1], it is important to decide if the activity is shared by both optical isomers and how it is influenced by N-alkylation.



(I)

We have determined (a) the maximum concentrations (of such compounds) tolerated by *ERK* cells (MTC's)[2], (b) the effectiveness of the compounds (at half MTC's) in delaying onset of cytopathic change in poliovirus-infected *ERK* cells[3], and (c) the concentrations of compound needed to produce various percentage reductions in poliovirus multiplication after 16 h incubation of *ERK* cells in the presence of both virus and compound[4].

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