Reinvestigation of the 4-Methoxy-2,6-dimethylbenzenesulphonyl (Mds) Protecting Group for the Guanidino-function during Peptide Synthesis

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Summary The procedure for the synthesis of Z-Arg(Mds)-OH yields, in addition to the title compound, three products that have analogues of the Mds group on the guanidino-function making characterization of protected intermediate peptides difficult.

OWING to its ease of removal by trifluoroacetic acid containing thioanisole,[1] the 4-methoxy-2,6-dimethylbenzenesulphonyl (Mds)[2] group was a welcome addition to the meagre number of groups suitable for protection of the arginine guanidino-function during peptide synthesis. However, in our hands, the procedure described for the preparation of 4-methoxy-2,6-dimethylbenzenesulphonyl chloride (Mds-Cl)[3] and its subsequent addition to Z-Arg-OH yielded not only Z-Arg(Mds)-OH, but also the analogue containing a 2-methoxy-4,6-dimethylbenzenesulphonyl group, a chlorine-containing analogue (most likely the 5-chloro-2-methoxy-4,6-dimethylbenzenesulphonyl group), and a small amount of a higher molecular weight arginine-containing compound that may be an analogue with two Mds groups.

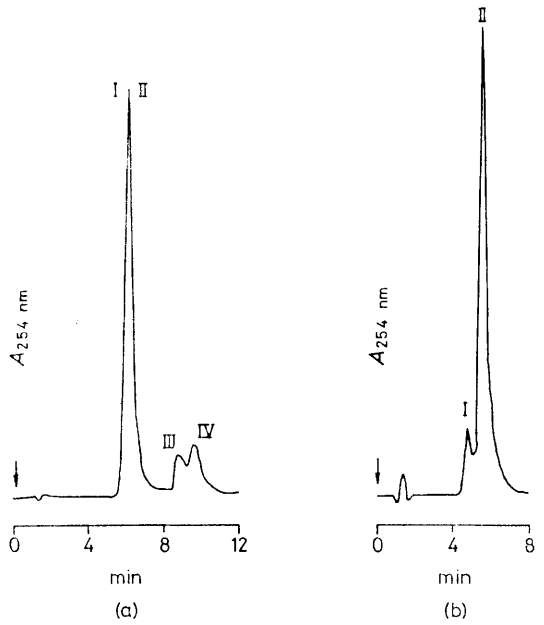

FIGURE. H.p.l.c. chromatography of Z-Arg(Mds)–OH on a 4·6 × 250 mm column of Merck LiChrosorb RP-18 (10 μm) eluted at 2 ml/min with (a) 0·1% aqueous trifluoroacetic acid–acetonitrile (3:2), (b) 0·1 M NaH_2PO_4 (pH 3)–acetonitrile (3:2).

Crude Z-Arg(Mds)-OH, isolated as its cyclohexylamine salt, had the same physical properties (m.p., optical rotation) as reported by Fujino *et al.*[2] and appeared to be homogeneous by t.l.c. Only after examination by analytical reverse-phase h.p.l.c. was the multiplicity of products evident. Chromatography in two systems revealed four components (Figure). Use of these solvent systems in preparative reverse-phase chromatography on a 37 × 350 mm column of Merck LiChroprep RP-18 (40—63 μm) allowed the separation and isolation of the compounds in peaks I, II, and IV. For the material from peak I the ^1H n.m.r. spectrum in $CDCl_3$ exhibited resonances associated with the guanidino-protecting group at δ 6·50 (2H, s), 3·70 (3H, s), 2·47 (3H, s), and 2·23 (3H, s). The non-equivalence of the protons of the two methyl groups and a mass spectrum for the methyl ester giving an m.w. of 520 combine to make a reasonable argument that the material in peak I is N^{α}-benzyloxycarbonyl-N^{G}-(2-methoxy-4,6-dimethylbenzenesulphonyl)arginine. Peak II material also has a mass spectrum for the methyl ester indicating an m.w. of 520, but the ^1H n.m.r. spectrum exhibits resonances for the guanidino-protecting group at δ 6·53 (2H, s), 3·73 (3H, s), and 2·57 (6H, s) as expected for Z-Arg(Mds)-OH. The mass spectrum for the methyl ester of the compound in peak IV showed a weak parent ion at m/z 554. The lactam formed by activation with dicyclohexylcarbodi-imide gave a strong parent ion (P) at m/z 522 with a ratio of P to $P + 2$ of (100:46) strongly suggesting the presence of a chlorine atom which was verified by elemental analysis. The ^1H n.m.r. spectrum showed resonances attributable to the guanidino-protecting group at δ 6·67 (1H, s), 3·70 (3H, s, 2·63 (3H, s), and 2·32 (3H, s). While there are a number of isomers that would accommodate the presence of a chlorine on the benzene ring and non-equivalent methyl groups, examination of the mechanism of chlorosulphonation[4] suggests the 5-chloro-2-methoxy-4,6-dimethylbenzenesulphonyl group as the most likely structure present in IV. Peak III material could not be isolated free of IV on a preparative scale, but enough of the methyl ester was isolated by analytical h.p.l.c. to attempt mass spectral analysis. Unfortunately, it gave a very poor mass spectrum, but did show ions with $m/z > 600$.

Identification of compounds present in crude Z-Arg(Mds)-OH points to the reaction of chlorosulphonic acid with 3,5-dimethylanisole as the source of difficulties. Addition of chlorine to aromatic compounds during chlorosulphonation has been previously reported[5] and the formation of 2-methoxy-4,6-dimethylbenzenesulphonyl chloride is easily rationalized. Indeed, ^1H n.m.r. spectroscopy of crude 4-methoxy-2,6-dimethylbenzenesulphonyl chloride showed not only the absence of starting material and the expected resonances at δ 6·67 (s), 3·83 (s), and 2·67 (s),[3] but also resonances at 6·92 (s), 6·80 (s), 3·96 (s), 2·72 (s), 2·58 (s), 2·42 (s), and 2·38 (s). Low volatility and high reactivity of the chlorosulphonate did not offer the option of purification. Neither were we successful in effecting an appreciable change in the ratio of products through adjustment to the reaction conditions (solvent, temperature, addition of NaCl).[4] We were able to obtain Z-Arg(Mds)-OH in >95% purity by high performance reverse-phase chromatography.

It should be emphasized that treatment of crude Z-Arg-(Mds)-OH under conditions to remove the Mds group does in fact result in the removal of all the various benzenesulphonyl groups present, so the purity of final products is unaffected. However, characterization of intermediate protected peptides is extremely difficult and the potential user of the Mds group should be wary.

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