New Procedure for Oxidation of 3-Substituted Indoles to Oxindoles: Modification of Tryptophan Residues in Peptides and Proteins

By W. E. SAVIGE and A. FONTANA*

(Institute of Organic Chemistry and Centro di Studio sui Biopolimeri del C.N.R., University of Padova, 35100 Padova, Italy)

Summary A new high-yield procedure for the oxidation to oxindoles of 3-substituted indoles, including tryptophancontaining peptides and proteins, involving the use of a mixture of dimethyl sulphoxide and concentrated HCl, is described.

WE report a new general method considerably superior to those in current use¹ for oxidation of 3-substituted indoles (1) into the corresponding oxindoles (2) using a mixture of dimethyl sulphoxide and concentrated HCl. The method gives high yields of conversion, not only for simple indoles, but also for tryptophan-containing peptides and proteins.

The oxidation can be effected by treating the indole compound with a mixture of Me_2SO (5—10 equiv.) and 12M HCl (10—20 equiv.) for 10—15 min at room temperature, the reaction then being stopped by addition of water.



If desired, the reaction can be carried out in the presence of a suitable solvent (up to 5 v/v) such as glacial acetic acid, trifluoroacetic acid, acetonitrile, or tetrahydrofuran, the reaction time then being extended up to 2 h. L-Tryptophan (2 mg) was treated with Me₂SO-HCl with slight variations in the above conditions and the reaction mixture was then analysed by gel filtration on a Sephadex LH-20 column or by automatic amino-acid analysis.[†] The results obtained indicate that the conversion of tryptophan (λ_{max} 280 nm) into oxindolylalanine (2-hydroxy-tryptophan² (λ_{max} 250 nm) can be made quantitative by a proper choice of the reaction conditions. Some dioxindolylalanine,³ presumably formed by further oxidation of oxindolylalanine, was noted under more drastic conditions, *i.e.*, greater excess of Me₂SO and longer reaction times. L-Tryptophan was also oxidised on a preparative level (100 mg) using the above conditions and pure L-oxindolylalanine was obtained in 70% yield, after recrystallization from water.

The reaction was further extended to tryptophancontaining polypeptides and proteins. The pentapeptide Phe-Val-Gln-Trp-Leu,⁴ corresponding to the 22—26 sequence of glaucagon, and valine-gramicidin A,⁵ an *N*formyl pentadecapeptide ethanolamide containing four tryptophan residues per molecule, were both treated with Me₂SO-HCl. Fractionation of the reaction mixture on Sephadex columns showed in both cases over 95% conversion into an oxindole compound. Hydrolysis of the oxidized peptides using 3M toluene-*p*-sulphonic acid⁶ and amino-acid analysis of the hydrolysates showed in both cases virtually quantitative conversion of tryptophan into oxindolylalanine.

Hen egg white lysozyme, a protein containing six tryptophan residues per molecule,⁷ was chosen as a test protein and treated analogously with Me₂SO-HCl for 15 min at room temperature. After dilution with water, the modified protein was separated on a Sephadex column and then

[†] Tryptophan and oxindolylalanine are eluted from the short column of the amino-acid analyser (D. H. Spackman, W. H. Stein, and S. Moore, *Analyt. Chem.*, 1958, **30**, 1190) just after the acidic and neutral and preceding the basic amino-acids. The oxindolylalanine peak precedes that of tryptophan. Dioxindolylalanine is eluted from the long column between tyrosine and phenylalanine.

hydrolysed with 3N toluene-p-sulphonic acid. Amino-acid analysis of the hydrolysate gave a figure of 6.2 (theory 6) oxindolylalanine residues per molecule. Methionine (2 residues per molecule) was quantitatively converted into methionine sulphoxide (found 1.9), while all other aminoacids, including cysteine, tyrosine, and histidine, were unchanged.[‡]

The results of the present work indicate that oxidation with Me₂SO-HCl provides a useful procedure for the modification of tryptophan in peptides and proteins. The reaction offers a new, practical alternative procedure to the available ones8 for the synthesis of oxindolylanine and its derivatives and should prove of value also in the oxidation of other indole compounds of biological and pharmacological interest.

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‡ Experiments with a mixture containing all common amino acids indicate that, in addition to tryptophan and methionine, cysteine is also oxidized to cystine. Ribonuclease, a tryptophan and cysteine-free protein, was oxidized by Me2SO-HCl only at the level of methionine residues.

¹ W. B. Lawson and B. Witkop, J. Org. Chem., 1961, 26, 263; R. L. Himman and C. P. Bauman, ibid., 1964, 29, 1206; T. Kobayashi and N. Inokuchi, Tetrahedron, 1964, 20, 2055; R. J. Sundberg, 'The Chemistry of Indoles,' Academic Press, New York-London, 1970, pp. 296-307; A. Fontana and C. Toniolo, in 'Progress in the Chemistry of Organic Natural Compounds,' eds. W. Herz, H. Griesebach, and and G. W. Kirby, Springer Verlag, Heidelberg-New York, 1976, vol. 33, p. 309.

- ² J. W. Cornforth, R. H. Conforth, C. E. Dalgliesh, and A. Neuberger, *Biochem. J.*, 1951, 48, 591. ³ P. L. Julian, E. E. Dailey, H. C. Printy, H. L. Cohen, and S. Hamashige, *J. Amer. Chem. Soc.*, 1956, 78, 3503.
- ⁴ E. Wünsch and F. Drees, Chem. Ber., 1967, 100, 816.
 ⁵ R. Sarges and B. Witkop, J. Amer. Chem. Soc., 1965, 87, 2011.
 ⁶ T. Y. Liu and Y. H. Chang, J. Biol. Chem., 1971, 246, 2842.

⁷ R. E. Canfield, J. Biol. Chem., 1963, 238, 2691. ⁸ Th. Wieland, O. Weiberg, W. Dilger, and E. Fisher, Annalen, 1955, 592, 69; F. M. Veronese, A. Fontana, E. Boccu', and C. A. Wieland, O. Weiberg, W. Dilger, and E. Fisher, Annalen, 1955, 592, 69; F. M. Veronese, A. Fontana, E. Boccu', and C. A. Benassi, Z. Naturforsch. (B), 1968, 32, 1319; M. Ohno, T. F. Spande, and B. Witkop, J. Org. Chem., 1974, 39, 2635.