Oxidation of Aromatic Substrates. Part 3.[1] Synthesis of Amino-acids by the Selective Action of Ruthenium Tetraoxide upon Arylalkylamines

By **David C. Ayres**, Chemistry Department, Westfield College, Hampstead, London NW3 7ST

The more strongly basic alkylamines are protected in dilute acid from oxidation by ruthenium tetraoxide, in contrast to aromatic amines which are degraded under the same conditions. One of the possibilities this offers for the selective oxidation of substituted alkylamines is illustrated by the degradation of arylalkylamines with cleavage of the aromatic ring. The only oxidation residue from the ring is a carboxy-group, which is incorporated with the retained amino-group in the product amino-acid. Yields are improved in the presence of electron-donating substituents which increase the rate of cleavage and so reduce the time of exposure of the product to further oxidation.

THE production of alkanoic acids from aromatic precursors by ruthenium tetraoxide depends upon the ease with which this reagent attacks π-bonded carbon atoms; for example the oxidation of phenylcyclohexane to give cyclohexanecarboxylic acid in 25% yield. Carboxylic acids are stable under the conditions employed, and it is probable that the yields obtained [2] are reduced by side reactions, initiated by attack at C_α of the alkylbenzene, or by chemisorption of the acid on precipitated ruthenium dioxide. The adaption of this reaction to amino-acid synthesis required protection of the amino-group in the side chain of the arylalkylamine. One method is the preparation of the trifluoroacetyl-amine and some applications of this are given in the following paper; in the present instance control was effected by dissolution of the substrate in an acid buffer. In earlier work [3] the less accessible alkanolamines were oxidised by traditional reagents in dilute sulphuric acid solution, where the amino-group was protected as its conjugate acid and amino-acids were isolated. Alkylamines (pK_a *ca.* 10.6) are stable for many hours at ambient temperature in dilute acid when in contact with a yellow solution of the tetraoxide in carbon tetrachloride. However, this protection is not afforded to aniline (pK_a 4.6) and its derivatives as the higher concentration of free base results in rapid oxidation under similar conditions; even in strong sulphuric acid (25% by weight) aniline sulphate reacted immediately in contact with RuO_4–CCl_4, with initial discolouration of the organic phase and subsequent precipitation of the black dioxide. *p*-Nitroaniline, one of the weakest nitrogen bases, is rapidly oxidised in 40% sulphuric acid solution. This marked disparity in oxidation rates in acid may be used as a qualitative test to distinguish between aliphatic and aromatic amines. For synthetic work with arylalkylamines we chose buffer solutions rather than sulphuric acid for better control of the degree of protonation of substrates and hence of the reaction rates.

The simplest application is to amino-acids in which the functional groups are set apart. This is shown by the oxidation of tyramine and of γ-(4-hydroxyphenyl)-propylamine to β-alanine (86% yield) and γ-amino-butyric acid (69%), respectively. Typical conditions involved use of a solution of the amine in phosphate buffer (pH 3.0) containing sodium periodate (12 equiv.) and a catalytic amount of ruthenium trichloride. No information about the products of aromatic ring cleavage by ruthenium tetraoxide is available and no attempt has been made here to identify them, but the oxygen demand is compatible with oxonolytic cleavage.[1]

$$+ 2(CO_2H)_2 + CO_2$$

Sodium hypochlorite is a cheaper and generally more convenient secondary oxidant but it could not be used under these acidic conditions. The separation of the less soluble iodate was however a useful guide to the progress of the reaction; and limitation of the primary oxidant reduces costs and avoids adsorption of product by ruthenium dioxide which is precipitated on completion. Although the oxidations may be completed within 30 min it is advisable to refrigerate the liquors and to allow time for the separation of salts before isolation of the amino-acids by passage through the acidic form of a sulphonic acid resin. Once elution of phosphate is complete the product is displaced with ammonium hydroxide.

Synthesis of the more important class of α-amino-acids in acceptable yields from benzylamine has also been achieved. In selecting benzylamines as precursors for α-amino-acids one must note the potential interaction between the nucleus and the amino-group. This is

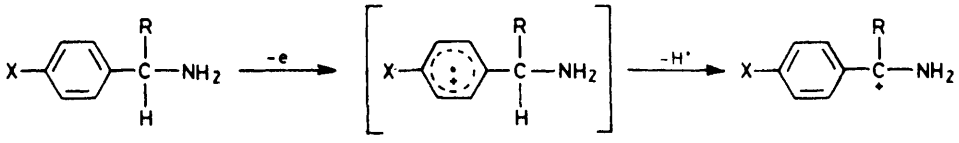
[1] Part 2, D. C. Ayres and A. M. M. Hossain, *J.C.S. Perkin I*, 1975, 707; *cf.* D. C. Ayres, *J.C.S. Chem. Comm.*, 1975, 440.

[2] J. A. Caputo and R. Fuchs, *Tetrahedron Letters*, 1967, 4729.

[3] J. H. Billmann, E. E. Parker, and W. T. Smith, *J. Biol. Chem.*, 1947, **180**, 29.

illustrated below and may necessitate adjustment of the conditions for individual synthesis. General guidelines are reported but in most instances the yields have not been optimised.

The oxidation of benzylamine was complete in 17 min at pH 4.5 and 20 °C but the yield of glycine was low. A test of glycine at pH 3.0 and 2 °C showed that it was little affected on contact with RuO_4–CCl_4 and at this temperature and pH the yield was increased to 60%. α-Phenylethylamine was then selected as a typical precursor for the synthesis of glycine homologues but under these conditions it gave only 10% of α-alanine. The principal stabilising feature is the extent of protonation of the amino-group and this increases as the acidity of the buffer diverges from the isoelectric pH (5.97 for glycine, 6.00 for α-alanine, and 6.90 for β-alanine [4]). The two-phase test was consistent with this view and showed that α-alanine was no less stable than glycine under the reaction conditions; hence the loss of material must be attributed to another cause. It is probable that a radical-cation participates,[5] and possible that



reactions in the side chain are encouraged by the tertiary carbon of the benzylamine homologues. This explanation is consistent with the behaviour of α-(4-methoxyphenyl)ethylamine, which afforded a 50% yield of α-alanine under standard conditions where the methoxy-substituent enhances the rate of ring fragmentation. Neither the separation of amine–periodate complexes [6] nor the oxidation of the amino-acids by periodate [7] is significant under the chosen conditions.

A qualitative guide to the relative rates of degradation of these precursors was obtained from a ^1H n.m.r. study of the reaction buffered in deuterium oxide. Accurate integration was not possible owing to the separation of ruthenium dioxide and the outgassing which occurred before the reaction was complete, but in the early stages the coincident disappearance of aromatic proton signals and the appearance of those attributed to amino-acids could be seen. Thus for α-(4-methoxyphenyl)ethylamine the double doublet centred at δ 7.26 (aromatic) and the singlet at δ 3.88 (OMe) were reduced to about one third of their intensity within 2 min of initiation by ruthenium trichloride at pH *ca.* 3.0; a doublet typical of α-alanine developed at δ 1.48.

It is to be expected that synthesis of α-alanine could be further improved by the choice of α-(hydroxyphenyl)-alkylamines as precursors, for like the alkoxy-compounds they are available in high yield by reduction [8] of the corresponding oxime. The single step conversion at pH 3.5 of L-tyrosine into L-aspartic acid in 60% yield illustrates this approach which is an improvement on existing multi-step procedures.[9] It clearly has potential for the correlation of the absolute configurations of aromatic compounds with those of aliphatic carboxylic acids, although the conditions were not optimised and considerable racemisation occurred. The c.d. curve of the crude aspartic acid was of the same form as that obtained from a pure reference sample with a common maximum at 205 nm, but in synthetic material the intensity was reduced to a quarter. Since L-aspartic acid is commonly isolated from strongly acidic solutions [10] it is unlikely to be affected by the buffer and racemisation is therefore most probably associated with the oxidation mechanism. The 60% yield obtained is acceptable as the operating pH was close to the isoelectric point of aspartic acid (p*I* 2.77).

A fifty-fold difference in the relative concentration of two free bases which differ in strength by two p*K* units can be established by a proper choice of the buffer pH. One can therefore envisage other selective oxidations of polyfunctional compounds where the oxidised and protected groups differ less in base strength than do those of the arylalkylamines.

EXPERIMENTAL

M.p.s were taken on a hot-stage apparatus. I.r. spectra (KBr discs) were recorded with a Perkin-Elmer 257 instrument and n.m.r. spectra were obtained with a Varian HA-100 spectrometer. Routine g.l.c. analysis were carried out with a Varian 1700 chromatograph and g.l.c.–mass spectrometric measurements were made with a Varian MAT 111 instrument.

Preliminary Oxidation Trials.—A clear yellow solution of ruthenium tetraoxide was prepared in carbon tetrachloride (40 ml) from ruthenium trichloride trihydrate (500 mg) and sodium hypochlorite (30 ml; 'Brobat' with 3.5% available chlorine). This can be kept indefinitely in a closed vessel in the presence of a little solid sodium periodate. It was washed with water before use and a sample (1 ml) placed in contact with the amine solution (1 ml of buffer or sulphuric acid containing *ca.* 5 mg of substrate), and the two phases were left undisturbed at 0 °C in stoppered tubes (0.5 in i.d.). The phosphate buffers in the pH range 3.0—4.5 were prepared according to Perrin and Dempsey [11]

[4] J. P. Greenstein and M. Winitz, 'The Chemistry of the Amino-Acids,' Wiley, New York, 1961, p. 496.
[5] D. C. Ayres and R. Gopalan, *J.C.S. Chem. Comm.*, 1976, 890.
[6] K. J. Jaura, K. K. Tewari, and R. L. Kaushik, *J. Indian Chem. Soc.*, 1963, **40**, 1008.
[7] J. R. Clamp and L. Hough, *Biochem. J.*, 1965, **94**, 17.
[8] O. Schales, *Ber.*, 1935, **68**, 1943.
[9] S. Goldschmidt and G. Freyss, *Ber.*, 1933, **66**, 784; P. A. Levene and S. Mardashew, *J. Biol. Chem.*, 1937, **117**, 179.
[10] Ref. 4, p. 1856.
[11] D. D. Perrin and B. Dempsey, 'Buffers for pH and Metal Ion Control,' Chapman and Hall, London 1974.

and the pH was checked after addition of substrate. The times which elapsed (see Table) before colour changes were detected gave an indication of the reactivity of some typical bases; darkening and precipitation of black ruthenium dioxide occurred when the oxidation was further advanced.

Qualitative observations of base oxidation rate variations with acidity in the two-phase system at 0 °C

Substrate	% H_2SO_4 (w/w) or buffer pH	Time from start	Colouration in CCl_4 layer
Aniline	60%	1 min	Red-brown
p-Toluidine	60%	1 min	Red-brown
Anisole	60%	Immediate	Violet
Aniline sulphate	40%	Few s	Red-brown
p-Nitroaniline	40%	Few s	Red-brown
Butylamine	40%	4 h	None
Benzylamine	40%	1 min	Red-brown
Glycine	40%	10 min	None
Heptylamine	pH 4.3	Few s	RuO_2 pptd.
1-Ethylpentylamine	4.3	7 min	Green
Pyridine	4.3	50 min	Discoloured
Glycine	4.3	5 min	Grey
Benzylamine	pH 3.0	12 min	Green
Glycine	3.0	30 min	None
α-Alanine	3.0	30 min	None
Valine	3.0	30 min	None
Aspartic acid	3.0	30 min	Discoloured

Sources of Amines and of Amino-acids.—L-Tyrosine used as a precursor and other amino-acids used as reference compounds were supplied by B.D.H. Biochemicals Ltd., and a sample of 4-(3-aminopropyl)phenol,[12] m.p. 102°, was kindly donated by Dr. D. J. Beames.

The oximes of acetophenone and 4′-methoxyacetophenone were obtained by addition of ketones in an equal volume of ethanol to acetate-buffered hydroxylamine hydrochloride (60% excess) in 2.5 times the volume of 7 : 3 water–ethanol. After 24 h almost quantitative yields were obtained and after washing with a little 65 : 35 water–ethanol these oximes were hydrogenated [8] in acidified ethanol to give α-phenylethylamine, b.p. 82° at 200 mmHg, and α-(4-methoxyphenyl)ethylamine, b.p. 64° at 0.2 mmHg, in *ca.* 80% yield. The products were characterised as their picrates, m.p. 189 and 178°, respectively.[8,13]

Synthesis of α-Alanine.—In this typical procedure α-(4-methoxyphenyl)ethylamine (498 mg, 3.3 mmol) was dissolved in the buffer (10 g of sodium dihydrogen phosphate in 50 ml of water; pH 2.4) containing 1% of the oxidation catalyst (18 mg of $RuCl_3,3H_2O$), followed by sodium periodate (8.4 g, 12 equiv. in 35 ml of water). At this point the solution pH was 3.7 and where necessary it may be lowered by addition of a small amount of phosphoric acid. After stirring at 2 °C for 10 min crystals of sodium iodate began to separate and the reaction was allowed to continue for a further 10 min before quenching with propan-2-ol (3.5 ml). Any residual amine may be detected by g.l.c. of a chloroform extract of the basified liquor. A 3% SE30–Chromosorb W (60—80 mesh) column operating with a nitrogen flow rate of 18 ml min^{-1} in the 75—90 °C temperature range was generally satisfactory, although no unchanged amine was detected under the above conditions of oxidation. T.l.c. on Kieselgel G using 80% ethanol–ammonia as eluant is an acceptable alternative method.

The mixture was refrigerated at 2 °C overnight before filtration of the liquor from insoluble sodium iodate; the filtrate was then charged on a column (*ca.* 30 cm long, 2 cm i.d.) of Dowes 50W (H^+) resin (60 g) in the acid form and eluted with water at about 3 ml min^{-1} to remove phosphate. The phosphomolybdate test [14] showed that this stage was complete once 200 ml of eluate had been collected and the amino-acid was then displaced [15] with ammonia solution (4N). Ninhydrin-positive material was concentrated in about 30 ml of alkaline liquor, which was evaporated under nitrogen to small bulk to remove ammonia before isolation of crude α-alanine (150 mg, 50%) by freeze-drying overnight.

Characterisation of Products.—All crude materials were compared with a standard amino-acid by paper chromatography (60% methanol–water as eluant).

Glycine, α-alanine, and β-alanine were purified by sublimation [16] at 170—190 °C and 0.15 mmHg and the i.r. spectra of the sublimates were identical with those of reference samples. If ammonia is not removed completely during evaporation of the eluate from the resin column an involatile residue of the ammonium salt will remain. α-Alanine was further characterised by g.l.c.–mass spectrometry of its *N*-trifluoroacetyl derivative; [17] this technique was most suitable for the involatile aspartic acid and for γ-aminobutyric acid. Thus the crude amino-acids were esterified by refluxing their solutions in Bu^nOH–HCl and treatment of the higher boiling residue in methylene chloride with trifluoroacetic anhydride at ambient temperature. A chromatography column (2 m by 2 mm i.d.) having a stationary phase (2%) of OV-17 (methyl : phenyl silicone as 55 : 50) was employed with a helium flow of 15 ml min^{-1}. There was a 6 min time delay at the initial temperature of 100 °C before temperature programming at 4° min^{-1}. Only single g.l.c. peaks were obtained from each of these crude products whilst their mass fragmentation patterns were unequivocal and included the following major ions:

butyl *N*-trifluoroacetyl-α-alaninate, *m/e* 141(31), 140 (100, M^+ − BuO·CO), 57(25)
butyl *N*-trifluoroacetyl-γ-butyrate, *m/e* 182(46, M^+ − BuO), 154(30, M^+ − BuO·CO), 140(7.5), 126(36)
dibutyl *N*-trifluoroacetylaspartate, *m/e* 268(2.5, M^+ − BuO), 240(86, M^+ − BuO·CO) 212(71), 198(35), 184(100), 140(84), 139(92, M^+ − 2BuO·CO)

The c.d. maximum of L-aspartic acid at 205 nm ($\Delta\varepsilon$ +1.11, 0.01%, in water) was recorded with the Cary 61 instrument.

N.m.r. Monitoring.—For the estimation of oxidation rate the amine (0.1 mmol) was dissolved in deuterium oxide (1 ml) containing sodium dihydrogen phosphate (245 mg) and sodium periodate (210 mg, 1 mmol or 10 equiv.). The pH was determined with short-range Universal paper and was brought to an indicated 2.5 units * by the addition

* Ref. 11, p. 82, gives pH = $pH_{obs.}$ +0.4.

[12] G. Goldschmidt and O. V. Fraenkel, *Monatsh.*, 1914, **35**, 383; H. W. Johnson and F. J. Gross, *J. Org. Chem.*, 1957, **22**, 1264.
[13] A. Michaelis and E. Know, *Ber.*, 1893, **26**, 2167.
[14] F. Feigel and V. Anger, ' Spot Tests in Inorganic Analysis,' Elsevier, New York, 6th edn., 1972, p. 388.
[15] M. E. Carston and R. K. Cannon, *J. Amer. Chem. Soc.*, 1952, **74**, 5950; M. E. Carston, *ibid.*, p. 5954.
[16] Ref. 4, p. 565.
[17] P. A. Cruickshank and J. C. Sheehan, *Analyt. Chem.*, 1964, **36**, 1191.

of 2 drops of acid (a solution of 0.2 g of phosphoric acid in 1 ml of D_2O). A slight deficiency of periodate delays iodate precipitation; however, some loss of performance from this cause is inevitable if solutions of acceptable concentration are to be prepared.

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