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Micellar Catalysis of Organic Reactions. XVII* Hydrolysis of Nitrazepam and Some *N*-Alkylated Derivatives

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Abstract

Product studies for the acid catalysed hydrolysis of nitrazepam and some *N*-alkyl derivatives in the presence of micelles of sodium dodecyl sulfate (sds) have been carried out by a u.v. spectrophotometric technique.

Attack of water at C 2 leading to initial amide cleavage is favoured by high acid concentrations, by micelles of sds and by small R groups attached to the amide nitrogen atom.

For nitrazepam, a change of mechanism from water attack at C 5 (leading to initial azomethine cleavage) to water attack at C 2 (leading to initial amide cleavage) was observed on transfer from water to micelles of sds. For *N*-benzyl nitrazepam (1d), however, no change of mechanism was detected. Initial attack of water occurred at C 5 (leading to initial azomethine cleavage), both in aqueous solution and in micelles of sds.

Introduction

There have been a number of reports[1–5] of the acid catalysed hydrolysis of therapeutically interesting benzodiazepinones (e.g., diazepam {1a} and nitrazepam {1b}) in aqueous solution. The diazepinone nucleus contains both azomethine [(4), (5)] and amide [(1), (2)] bonds. Complete hydrolysis of benzodiazepinones results from the sequential cleavage of both of these bonds forming the appropriate substituted 2-aminobenzophenone (4) and glycine (Scheme 1).

Initial attack of water at C2 results in amide cleavage and the production of intermediate (2), whereas initial attack of water at C5 results in azomethine cleavage and the production of intermediate (3). Subsequent decomposition of both intermediates (2) and (3) leads to the substituted 2-aminobenzophenone (4) and glycine.

In acidic solution, intermediate (2) does not accumulate due to the facile recyclization (2) → (1) and subsequent decomposition (2) → (4).

However, intermediate (3) does accumulate in acidic solution because the recyclization (3) → (1) is inhibited by protonation of the primary amino group

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[1] Han, W. W., Yakatan, G. J., and Maness, D. D., *J. Pharm. Sci.*, 1976, **65**, 1198.
[2] Han, W. W., Yakatan, G. J., and Maness, D. D., *J. Pharm. Sci.*, 1977, **66**, 573.
[3] Han, W. W., Yakatan, G. J., and Maness, D. D., *J. Pharm. Sci.*, 1977, **66**, 795.
[4] Nakano, M., Inotsume, N., Kohri, N., and Arita, T., *Int.. J. Pharm.* 1979, **3**, 195.
[5] Inotsume, N., and Nakano, M., *J. Pharm. Sci.*, 1980, **69**, 1331.

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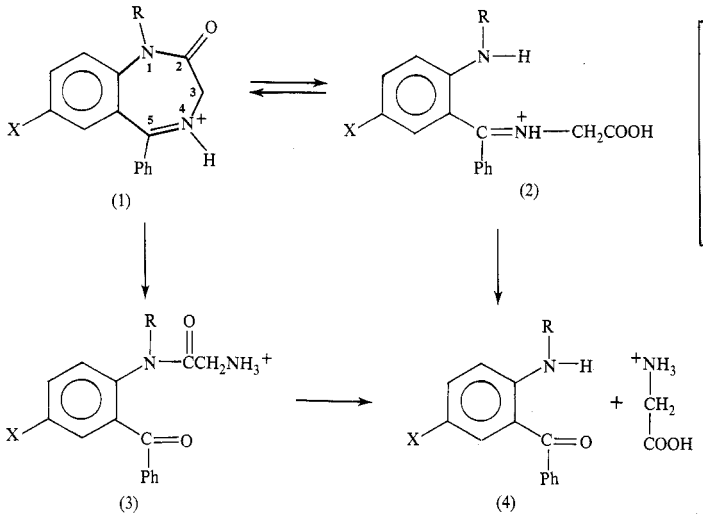
of (3) and because subsequent decomposition (3) → (4) is very slow. Thus, initial hydrolysis of the amide bond results in monophasic kinetics, while initial azomethine hydrolysis results in biphasic kinetics.

Hydrolysis of diazepam[2] (1a) and nitrazepam[3] (1b) in aqueous acidic solution has been reported to involve initial azomethine cleavage and the mechanism (1) → (3) → (4) was proposed. On the other hand, hydrolysis of oxazepam,[2] chlordiazepoxide[1] and demoxepam[1] has been reported to involve initial amide cleavage.

Thus, the mechanism of hydrolysis of benzodiazepinones is finely balanced and variation of substituents X and R can result in a change of mechanism. Because of our interest in the effects of micelles on the mechanism of reaction,[6,7] we chose to study the acidic hydrolysis of benzodiazepinones in the presence of micelles of sodium dodecyl sulfate (sds).[8]



	X	R
(a)	Cl	Me
(b)	NO_2	H
(c)	NO_2	CH_3
(d)	NO_2	CH_2Ph
(e)	NO_2	C_6H_{13}
(f)	NO_2	$C_{12}H_{25}$

Scheme 1

The acid catalysed hydrolysis of diazepam was inhibited by sds, but the mechanism was unchanged (initial attack of water at C5 leading to azomethine cleavage). The acid-catalysed hydrolysis of nitrazepam however was not inhibited by micelles of sds and the mechanism changed from initial attack of water at C5 (azomethine cleavage) in aqueous solution to initial attack of water at C2 (amide cleavage) in the presence of micelles of sds.[8]

We now report further results which confirm this mechanistic change for nitrazepam hydrolysis and also results for the hydrolysis of several *N*-alkyl derivatives of nitrazepam (1c)–(1f).

Results and Discussion

Diazepam (1a) and nitrazepam (1b) have two obvious differences, either one of which may lead to their different hydrolytic mechanisms in micellar solution. In an attempt to probe reasons for the mechanistic change by nitrazepam but not by

[6] Broxton, T. J., Fernando, D. R., and Rowe, J. E., *J. Org. Chem.*, 1981, **46**, 3522.
[7] Broxton, T. J., and Duddy, N. W., *Aust. J. Chem.*, 1979, **32**, 1717.
[8] Broxton, T. J., Ryan, T., and Morrison, S. R., *Aust. J. Chem.*, 1984, **37**, 1895.

diazepam, a number of *N*-alkylated nitrazepam derivatives (1c–f) were examined. To determine whether initial water attack was at C 2 (leading to amide hydrolysis) or at C 5 (leading to azomethine hydrolysis), the u.v. spectrum of the reaction mixture was recorded after 30 min of reaction.

Three components were detected in most mixtures. Under these conditions (68°C/30 min/various [HCl]), starting material (1), intermediate (3) and product (4) were present. It was shown independently that under the reaction conditions intermediate (3) was not converted into product (4) to any detectable extent. Thus, any product present must have arisen from initial amide hydrolysis [route (1) → (2) → (4)]. The extent of initial amide attack and initial azomethine attack was thus indicated by the ratio of product (4) to intermediate (3).

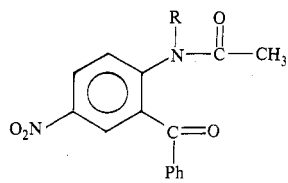
To determine the amount of each compound present in the three component mixtures, the absorbance of the ultraviolet–visible spectrum of the mixture was determined at three different wavelengths [e.g., 260, 280 and 380 nm for compound (1c)]. Provided the extinction coefficients of each component at each wavelength were known, three simultaneous equations could be derived, from which the concentration of each component of the mixture was calculated.

Table 1. Wavelengths used to analyse hydrolysis mixtures

Compound	R	[sds] (mM)	Wavelengths (nm)[A]	Compound	R	[sds] (mM)	Wavelengths (nm)[A]
(1b)	H	0	265, 280, 365	(1d)	CH_2Ph	0	260, 280, 380
		200	265, 280, 360			100	260, 280, 380
(1c)	Me	200	260, 280, 380	(1e)	C_6H_{13}	100	260, 280, 380
				(1f)	$C_{12}H_{25}$	80	260, 285, 380

[A] The lowest wavelength 260 or 265 nm, corresponds to λ_{max} of intermediate (2). The middle wavelength 280 or 285, corresponds to λ_{max} of reactant (1). The highest wavelength corresponds to the λ_{max} of the product (4). Since each component, (1), (2) and (4), absorbs at each wavelength, it was necessary to calculate the extinction coefficient of each component at each wavelength.

Ultraviolet spectra of reactants (1c–f) and products (4c–f) were obtained and extinction coefficients calculated at appropriate wavelengths (Table 1). Since the intermediate (3) was difficult to isolate and purify, a model compound (5), differing only in the replacement of a hydrogen atom by a NH_3^+ group, was used to determine the extinction coefficients of intermediates (3).



(5b) R = H
(5c) R = CH_3
(5d) R = CH_2Ph
(5e) R = C_6H_{13}
(5f) R = $C_{12}H_{25}$

It is unlikely that the NH_3^+ group on the side chain would significantly affect the u.v. spectrum of compound (3) since the u.v. spectrum is mainly influenced by the aromatic rings and the groups directly attached to them. Thus, we assume that the u.v. spectra of, for example, (3d) would be very similar to that of (5d). Some support for this assumption is available in the literature.[4,8] Extinction coefficients for model compounds (5c–f) were measured at the appropriate wavelengths (see Table 1).

From the extinction coefficients and the ultraviolet–visible spectra for the reaction mixtures, the concentrations of reactant, intermediate and product were calculated for each compound for a range of acid concentrations. The results of these product analyses are in Table 2.

Table 2. Product analyses after 30 min reaction time for the acidic hydrolysis of compounds (1b–f) at 68°C in the presence of sds, and for compounds (1b) and (1d) in water

Component[A]	[HCl] (M)	$10^5 \times$component (M) for compound (1b)[B]	(1b)	(1c)	(1d)[B]	(1d)	(1e)	(1f)
(1)	0·47	0·9	0·65	1·3	2·2	2·2	1·45	1·75
(3)		1·5	0	0·55	1·1	0·9	1·0	0·5
(4)		0·95	3·25	1·45	0·2	0·6	0·8	0·7
(1)	0·12	1·0	1·9	1·8	1·95	2·8	2·25	2·5
(3)		2·7	0	0·8	0·9	0·65	0·95	0·65
(4)		0·05	1·2	0·65	0	0	0·4	0·5
(1)	0·015	0·15	2·65	2·3	2·5	2·75	2·1	1·2
(3)		2·8	0·2	1·1	0·95	0·4	0·8	2·4
(4)		0·05	0·25	0·1	0	0·05	0·1	0

[A] (1), reactant; (3), open chain intermediate formed from azomethine hydrolysis; (4), products formed by initial amide hydrolysis.
[B] Results for reaction in water.

From Table 2 it can be seen that for nitrazepam (1b), hydrolysis at 0·12 M HCl resulted in formation of intermediate (3b) in water (i.e., initial azomethine cleavage) but formation of product (4b) in the presence of micelles of sds (i.e., initial amide cleavage). This confirms the conclusions reached in the previous paper in this series.[8]

Of the compounds studied, the *N*-benzyl derivative (1d) was the most interesting. At 0·12 M HCl, hydrolysis of the benzyl compound gave only intermediate (3d), whether in water or in micelles of sds. Thus, the presence of a benzyl substituent (R) in compound (1d) is sufficient to completely change the mechanism of hydrolysis in sds. For nimetazepam (1c; R = Me), the *N*-hexyl (1e) and *N*-dodecyl (1f) compounds, hydrolysis in the presence of micelles of sds resulted in concurrent amide and azomethine cleavage.

The percentage of initial amide hydrolysis for these compounds at 0·12 M HCl in the presence of micelles of sds is given in Table 3.

One possible explanation for the observed variation in the extent of initial amide cleavage (i.e., attack by water at C2) is steric hindrance by the substituent R to the attack of a water molecule. As the steric bulk of the substituent R, as measured by Taft E_s values (Table 3),[9] is increased, the percentage of reaction occurring with attack of water at C2 decreases. This cannot, however, be the complete answer, because the *N*-hexyl and *N*-benzyl compounds which have very similar E_s values, lead to quite different percentages of amide hydrolysis (Table 3).

Another steric factor worth considering is the hindrance provided by the micelle itself as the reaction centre is drawn closer to the micelle surface. If we assume

[9] Taft, R. W., Jr, in 'Steric Effects in Organic Chemistry' (Ed. M. S. Newman) p. 598 (John Wiley: New York 1956).

that the interior of the micelle is water free,[10,11] then as the amide carbonyl group is drawn into the micelle, attack of water must be increasingly difficult. As an indication of the attraction of the micelle for the substrate molecule, we could consider the hydrophobicity of the alkyl group R. Compounds with hydrophobic R groups would almost certainly be strongly bound to the micelle, probably with a radial orientation. This would lead to the hydrophobic R group being buried into the micelle and the protonated azomethine group being directed into the aqueous intermicellar pseudophase. A useful measure of the hydrophobicity of the substituent R is obtained from the hydrophobic fragmental constant[12] (Table 3). As with steric effects, hydrophobicity is not the complete answer to the variation in the percentage of initial amide hydrolysis. This is exemplified by the similar results obtained for compounds containing groups of such widely differing hydrophobicity as the *N*-methyl and *N*-dodecyl compounds.

Table 3. Derived data for the acid-catalysed hydrolysis of *N*-alkylated nitrazepam derivatives in the presence of sds at 68°C

Substituent	Amide hyd. (%)	Taft E_s value	f(octanol)[A]
H	100	1·24	
Me	45	0	0·702
$C_{12}H_{25}$	43	[B]	6·53
C_6H_{13}	30	−0·40	3·35
CH_2Ph	0	−0·38	2·42

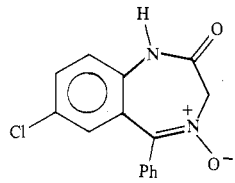
[A] Hydrophobic fragmental constant.
[B] Unknown, but probably similar to the value for the n-hexyl compound. n-Alkyl groups with three or more carbon atoms have E_s values between −0·33 and −0·40 (ref. 9).

From these results (Table 2), it seems safe only to conclude that the introduction of an alkyl group R in the nitrazepam nucleus hinders attack of water at C2 in the presence of micelles of sds. At higher acid concentrations (0·47 M HCl), in sds attack of water at C2 is more prevalent than at lower (e.g., 0·12 M HCl) acid concentrations for compounds (1c), (1d), (1e) and (1f). In fact, at 0·47 M HCl, there is even some evidence of attack of H_2O at C2 in aqueous solution [about 40% for nitrazepam (1b)] but this is decreased to 15% by the introduction of the benzyl group in compound (1d).

Thus, attack of water at C2 leading to initial amide hydrolysis is favoured by high acid concentrations, by micelles of sds and by small (e.g., H) R groups. For example, in aqueous acidic solution,[1] the hydrolysis of demoxepam (6), which has only a hydrogen atom on the amide nitrogen, occurs predominantly via initial amide hydrolysis. It appears that the nature of the R group is more important in determining mechanism than the nature of the X group. For nitrazepam (R = H), initial amide hydrolysis is favoured by the addition of micelles of sds (e.g., for X = NO_2), even if it is not the preferred pathway in water. For demoxepam (6), initial amide cleavage is favoured even in the absence of micelles. The introduction of a methyl group on

[10] Dill, K. A., Koppel, D. E., Cantor, R. S., Dill, J. D., Bendedouch, D., and Chen, S.-H., *Nature (London)*, 1984, **309**, 42.
[11] Bendedouch, D., Chen, S.-H., and Koehler, W. C., *J. Phys. Chem.*, 1983, **87**, 153.
[12] Rekker, R. F., 'The Hydrophobic Fragmental Constant' (Elsevier: Amsterdam 1977).

the amide nitrogen, e.g. in diazepam (1a), however, results in no detectable initial amide cleavage, even in the presence of micelles of sds. Similarly, the mechanism of hydrolysis of nimetazepam (1c), is not as susceptible to change as that of nitrazepam to the addition of micelles of sds (Table 3).



(6)

Experimental

Materials

Nitrazepam (1b) and nimetazepam (1c) were provided by Roche Products Pty Ltd.

N-Alkylnitrazepam derivatives were prepared by the alkylation of nitrazepam in dimethylformamide at 0°C in the presence of sodium hydride and the appropriate alkyl halide. Excess reagents were evaporated under reduced pressure and the residue was purified by column chromatography (SiO_2/CH_2Cl_2). The compounds were characterized by ^{13}C and ^1H n.m.r. spectroscopy and either elemental analysis or by accurate molecular weight determination by high resolution mass spectrometry, where compounds were not sufficiently stable to get combustion analyses. Splitting was observed in the n.m.r. spectra of *N*-alkylated derivatives (1c–f), presumably because of restricted rotation due to hindering the freely changing conformational states of the seven membered ring. Similar problems were also observed for the model compounds (5c–f) and for some of the products of hydrolysis (4c–e). The splitting caused by restricted rotation in these compounds was lost by increasing the temperature causing the multiplets of doublets to collapse to singlets.[13] The N-*benzyl compound* (1d) had m.p. 168–169° (Found: $M^{+\bullet}$, 371·125. $C_{22}H_{17}N_3O_3$ requires $M^{+\bullet}$, 371·127). ^1H n.m.r. δ 3·69, d; 4·72, d, 2H, $NCH_2C{=}O$; 4·69, d; 5·53, d, 2H, NCH_2Ph; 7·1–7·4, m, 10H; 7·70, d, *J* 10 Hz, H 9; 8·08, d, *J* 3 Hz, H 6; 8·27, 8·31, dd, $J_{8,9}$ 10 Hz, $J_{6,8}$ 3 Hz, H 8. The N-*hexyl compound* (1e) had m.p. 91–93° (Found: $M^{+\bullet}$, 365·175. $C_{21}H_{23}N_3O_3$ requires $M^{+\bullet}$, 365·174). ^1H n.m.r. δ 0·81, t, 3H, terminal CH_3; 1·3–1·65, m, 8H, $4\times CH_2$; 3·69, d, 4·69, d, 2H, $NCH_2C{=}O$; 3·65, m, 4·30, m, NCH_2; 7·38, m, 6H; 8·08, d, *J* 3 Hz, H 6; 8·27, 8·31, dd, $J_{8,9}$ 10 Hz, $J_{6,8}$ 3 Hz, H 8. The N-*dodecyl compound* (1f) had m.p. 66·5–67·5° (Found: $M^{+\bullet}$, 449·268. $C_{27}H_{35}N_3O_3$ requires $M^{+\bullet}$, 449·267). ^1H n.m.r. δ 0·81, t, 3H, terminal CH_3; 1·3–1·7, m, 20H, $10\times CH_2$; 3·69, d, 4·69, d, 2H, $NCH_2C{=}O$; 3·65, m, 4·30, m, 2H, NCH_2; 7·38, m, 6H; 8·08, d, *J* 3 Hz, H 6; 8·27, 8·31, dd, $J_{8,9}$ 10 Hz, $J_{6,8}$ 3 Hz, H 8.

Model compounds (5b–f) were prepared from 2-amino-5-nitrobenzophenone by acetylation (Ac_2O/HOAc) and alkylation ($NaH/RBr/HCONMe_2$). Purification of the *benzyl compound* (5d) was achieved by column chromatography (SiO_2/CH_2Cl_2–C_6H_{14} 4 : 1), m.p. 125–126° (Found: $M^{+\bullet}$, 374·124. $C_{22}H_{18}N_2O_4$ requires $M^{+\bullet}$, 374·127). ^1H n.m.r. δ 1·95, s, 3H, CH_3CO; 4·1, d, 5·1, d, 2H, CH_2Ph; 7·5, m, 10H; 8·1, m, 3H. The methyl (5c), hexyl (5e) and dodecyl (5f) compounds suffered deacylation during the alkylation procedure. Reacetylation (HOAc/Ac_2O) yielded the required compounds (5c), (5e) and (5f), which were characterized by ^{13}C and ^1H n.m.r. spectroscopy. The *methyl compound* (5c) had m.p. 99–100° (Found: C, 64·4; H, 4·6; N, 9·3. $C_{16}H_{14}N_2O_4$ requires C, 64·4; H, 4·8; N, 9·4%). ^1H n.m.r. δ 1·9, s, 2·0, s, CH_3CO; 3·14, d, 3H, NCH_3; 7·49, m, 6H; 8·26, m, 2H. The *hexyl compound* (5e) was obtained as an oil pure by t.l.c. (silica/CH_2Cl_2) (Found: $M^{+\bullet}$, 368·169. $C_{21}H_{24}N_2O_4$ requires $M^{+\bullet}$, 368·174). ^1H n.m.r. δ 0·8, t, 3H, terminal CH_3; 0·8–1·8, m, 8H, $4\times CH_2$; 2·0, s, 3H, CH_3CO; 3·0, m, 2H, NCH_2; 7·5, m, 6H; 8·3, m, 2H. The *dodecyl compound* (5f) was obtained as an oil pure by t.l.c. (SiO_2/CH_2Cl_2) (Found: $M^{+\bullet}$, 452·263. $C_{27}H_{36}N_2O_4$ requires $M^{+\bullet}$, 452·268). ^1H n.m.r. δ 0·8, t, 3H, terminal CH_3; 0·9–1·8, m, 20H, $10\times CH_2$; 2·0, d, 3H, CH_3CO; 2·9, d, 2H, NCH_2; 7·6, m, 6H.

[13] Pople, J. A., Schneider, W. G., and Bernstein, H. J., 'High Resolution Nuclear Magnetic Resonance' (McGraw–Hill: New York 1959).

2-Acetylamino-5-nitrobenzophenone (5b) was available from previous studies.[8]

The 2-alkylamino-5-nitrobenzophenones (4c–f) were prepared by the hydrolysis of the appropriate *N*-alkyl nitrazepam derivative in 1 M HCl for 16 h at 70°. The amines were extracted ($CHCl_3$); the extract was washed (H_2O), dried ($MgSO_4$) and evaporated to dryness under reduced pressure. The amines were then purified by column chromatography (SiO_2/CH_2Cl_2) and characterized by ^1H n.m.r. and mass spectrometry.

2-Amino-5-nitrobenzophenone (4b) and 2-methylamino-5-nitrobenzophenone (4c) were commercially available (Aldrich).

The *benzyl compound* (4d) had m.p. 134·5–135·5° (Found: $M^{+\bullet}$, 332·114. $C_{20}H_{16}N_2O_3$ requires $M^{+\bullet}$, 332·116). ^1H n.m.r. δ 4·6, d, 2H, NCH_2; 6·7, d, $J_{3,4}$ 10 Hz, H 3; 7·0–7·8, m, 10H; 8·1, 8·2, dd, $J_{3,4}$ 10 Hz, $J_{4,6}$ 3 Hz, H 4; 8·5, d, $J_{4,6}$ 3 Hz, H 6.

The *hexyl compound* (4e) had m.p. 66·0–67·0° (Found: C, 69·9; H, 6·7; N, 8·7. $C_{19}H_{22}N_2O_3$ requires C, 69·9; H, 6·8; N, 8·6%). ^1H n.m.r. δ 0·9, t, 3H, terminal CH_3; 1·2–1·8, m, 8H, $4\times CH_2$; 3·4, m, 2H, NCH_2; 6·7, d, $J_{3,4}$ 10 Hz, H 3; 7·6, m, 5H; 8·2, 8·3, dd, $J_{3,4}$ 10 Hz, $J_{4,6}$ 3 Hz, H 4; 8·5, d, $J_{4,6}$ 3 Hz, H 6.

The dodecyl compound (4f) was prepared in the same way but it was not able to be purified. The hexyl compound (4e) was used instead for the u.v. spectral analysis as it should have a very similar u.v. spectrum to that of (4f).

Sodium dodecyl sulfate (BDH Biochemicals) was purified by the method of Duynstee and Grunwald.[14] Distilled water was further purified by a Millipore system, to achieve a resistivity of, at least, 10 MΩ cm.

Product Analysis

Stock solutions (1×10^{-2} M) of all the species studied (1b–f, 4b–e and 5b–f) were prepared in dry dioxan.

Stock solutions of sds (0·4 M) and HCl (1 M) were prepared in purified water. The aqueous acid and sds/acid solutions used for the product analyses were prepared by appropriate dilution of the stock solutions of HCl and sds. The solutions (3·5 ml) were placed into cuvettes and allowed 30 min in the constant temperature cell holder of a Varian 635 ultraviolet–visible spectrophotometer to reach thermal equilibrium. The temperature in the cuvette was measured by means of a Jenco thermistor thermometer. Then a sample (12 μl) of the stock solution of the required compound was added to the cuvette, the solution was shaken and the u.v. spectrum recorded on a National VP 6511A X-T recorder. Absorbances were read directly from the u.v. machine at the required wavelengths (Table 1). The extinction coefficients were calculated by use of Beer's law.

Product studies were carried out as above by scanning the u.v. spectrum and recording the absorbance of the desired wavelengths 30 min after the addition of 12 μl of the stock solution of the required reactant (1b–f). From the absorbance at each wavelength and the extinction coefficient of each species at that wavelength, three simultaneous equations could be obtained. Solution of these equations gave the results in Table 2.

Tests with a prepared three component mixture (1b, 4b, 5b) showed that this method was accurate to within 10%.

Acknowledgments

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[14] Duynstee, E. F. J., and Grunwald, E., *J. Am. Chem. Soc.*, 1959, **81**, 4540.