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Isolation and characterisation of an acid-catalyzed intermediate hydrolysis product of nitrazepam

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Summary

A method is described for the isolation in high yield and purity of an intermediate acid hydrolysis product of nitrazepam. The compound is obtained in maximum yield by heating a solution of nitrazepam in 0.1 M hydrochloric acid at 80 °C for 45 min and is isolated by a fast solvent extraction procedure to minimise further hydrolysis to 2-amino-5-nitrobenzophenone. Structural elucidation by infrared spectrometry, proton magnetic resonance spectrometry and elemental analysis has confirmed that the product is 2-glycylamino-5-nitrobenzophenone formed by cleavage of the 4,5-azomethine bond of nitrazepam.

Introduction

Hydrolysis of nitrazepam in aqueous solution yields 2-amino-5-nitrobenzophenone by cleavage of the 1,2-amide and 4,5-azomethine bonds. The reaction has been reported to proceed in acidic solution via the reversible formation of an intermediate formed by initial cleavage at the 4,5-bond.

This product has been identified though not conclusively as 2-glycylamino-5-nitrobenzophenone (Han et al., 1977). Spectral evidence for the structure assignment is equivocal: the yellow product was isolated by preparative TLC using a solvent system containing 7.4 M ammonium hydroxide which on theoretical grounds would be expected to cause recyclisation to nitrazepam. Its mass spectrum gave a putative molecular ion at the m/z value for the intermediate, and the nuclear magnetic resonance spectrum, of which no details were provided, was used only to confirm the absence of a carboxylic acid group.

The success of a fast solvent extraction technique for isolating an intermediate from alkaline hydrolysis (Davidson et al., 1990) prompted an exploration of its use in acid hydrolysis also.

Experimental

Nitrazepam was a gift from Roche Products Ltd. All reagents and solvents (BDH Chemicals) were of analytical reagent quality.

Infrared, ultraviolet and mass spectra were obtained as previously described (Davidson et al., 1990). The proton magnetic resonance spectra in deuterated chloroform were recorded by using a Brucker WN-250 spectrometer.

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Isolation of the acid-catalyzed product

Nitrazepam (1 g) dissolved in methanol (25 ml) and 0.1 M hydrochloric acid (200 ml) preheated to 80 °C was held thus for 45 min. The cooled solution was extracted with chloroform (3 × 100 ml) to remove 2-amino-5-nitrobenzophenone and unhydrolysed nitrazepam. The aqueous phase was transferred to a separating funnel containing 1 M sodium hydroxide (20 ml), 0.5 M Tris buffer pH 8 (50 ml) and chloroform (100 ml) and immediately shaken for 30 s. After 2 min the chloroform layer was evaporated to a small volume and the colourless crystals (900 mg) were recrystallised from chloroform (yield 500 mg).

Results and Discussion

Previous workers have reported that the intermediate acid hydrolysis product of nitrazepam (initial fission at the 4,5-bond) could not be isolated by solvent extraction owing to rapid recyclization to nitrazepam at pH values above the pK_a of the product. Such values are necessary to ensure that the aliphatic amino group is in the non-protonated state and that the product is extractable into chloroform (Inotsume and Nakano, 1980). However, the fast extraction technique used after alkali hydrolysis of nitrazepam (Davidson et al., 1990) has been found to be suitable. Nitrazepam was hydrolysed in 0.1 M hydrochloric acid (pH 1, 80 °C, 45 min): 2-amino-5-nitrobenzophenone and any unchanged nitrazepam were then extracted into chloroform. The aqueous layer was adjusted to pH 8 to convert the protonated intermediate product, at least partially, to its non-protonated form, and simultaneously extracted into chloroform. The colourless solid obtained by evaporation of the chloroform was shown to be free of 2-amino-5-nitrobenzophenone by the absence of colour or an absorption band at 350 nm. In contrast, the product isolated by Han et al., (1977) was described as a yellow solid. Conditions for the formation of the intermediate product (in the pH range 1–4) and its recovery by the fast extraction technique (in the pH range 4–10) were optimised by using procedures already described (Davidson et al., 1990).

Structure elucidation

The following data are consistent with the isolated product being 2-glycylamino-5-nitrobenzophenone.

Infrared spectrophotometry Band assignments (potassium bromide disc) are as follows:

Wavenumber (cm^{-1})		*Vibration mode*
3 403	N-H	asym. stretch of aliphatic primary amine
3 345	N-H	sym. stretch of aliphatic primary amine
1 695	C = O	stretch of aromatic ketone
1 640	C = O	stretch (amide I band)
1 610	N-H	bend of aliphatic primary amine
1 570	N-H	bend (amide II band)
1 492	NO_2	stretch asym.
1 333	NO_2	stretch sym.
1 080	C-N	stretch of aliphatic primary amine
820 (broad)	NH_2	twisting and wagging deformations of aliphatic primary amine

Mass spectrometry The high-resolution mass spectrum obtained by direct insertion on a probe operated at 150 °C and with an ionisation electron energy beam at 70 eV, gave a molecular ion at *m/z* 299.0915 (11.8% relative abundance) corresponding to $C_{15}H_{13}N_3O_4$ (theoretical *m/z* 299.0906). Although this molecular formula is common to both intermediates formed by initial scission at the 1,2- or 4,5-bonds of nitrazepam, the fragments (Table 1) confirm the structural formula as 2-glycylamino-5-nitrobenzophenone. The base peak at *m/z* 30.0338 due to the $CH_2NH_2^+$ fragment (theoretical *m/z* 30.0344) and the fragment at *m/z* 269.0561 (theoretical *m/z* 269.0562) due to the $C_{14}H_9N_2O_4^+$ fragment indicate α-cleavage with charge retention on either of the fragments.

Proton magnetic resonance spectrometry ^1H NMR (250 MHz, $CDCl_3$): δ 1.68 (2H, s, exchangeable with D_2O, NH_2); 3.58 (2H, s, CH_2);

TABLE 1

Principal m/z values in the mass spectrum of the isolated product

m/z [a]	Formula	Relative abundance (%)	Fragmentation
299	$C_{15}H_{13}N_3O_4$	11.8	M^+
281	$C_{15}H_{11}N_3O_3$	35.9	M^+-H_2O
280	$C_{15}H_{10}N_3O_3$	30.5	M^+-H_2O-H
271	$C_{14}H_{11}N_2O_4$	9.2	M^+-CH_2N
270	$C_{14}H_{10}N_2O_4$	41.2	M^+-CH_3N
269	$C_{14}H_9N_2O_4$	14.4	M^+-CH_4N
254	$C_{14}H_{10}N_2O_3$	14.8	M^+-H_2O-HCN
253	$C_{14}H_9N_2O_3$	45.2	M^+-H_2O-HCN-H
252	$C_{14}H_{10}N_3O_2$	13.5	M^+-H_2O-CO-H
252	$C_{14}H_8N_2O_3$	16.7	M^+-H_2O-HCN-2H
242	$C_{13}H_{10}N_2O_3$	24.6	M^+-$COCHNH_2$
241	$C_{13}H_9N_2O_3$	33.8	M^+-$COCH_2NH_2$
226	$C_{13}H_{10}N_2O_2$	25.3	M^+-H_2O-CO-HCN
105	C_7H_5O	27.1	M^+-$C_6H_5NHCOCH_2NH_2$
30	CH_4N	100	$CH_2NH_2^+$

[a] Only the integral m/z values are reported. The accurate values, determined to six places of decimals, agree with the calculated mass of the proposed structure to within 20 ppm.

7.54 (2H, t, $J = 7$ Hz, H-3′, H-5′); 7.70 (1H, m, H-4′); 7.76 (2H, d, $J = 7$ Hz, H-2′, H-6′); 8.42 (1H, dd, $J = 9$ and 2 Hz, H-5); 8.45 (1H, d, $J = 2$ Hz, H-3); 8.98 (1H, d, $J = 9$ Hz, H-6); 12.14 (1H, s, exchangeable with D_2O, NH).

Elemental analysis Found C, 60.16; H, 4.15; N, 14.01. $C_{15}H_{13}N_3O_4$ requires C, 60.20; H, 4.38; N, 14.04.

Ultraviolet spectrophotometry Maximum absorption at 264 nm ($\epsilon = 1.87 \times 10^4$) was obtained in agreement with Inotsume and Nakano (1980) for authentic 2-glycylamino-5-nitrobenzophenone.

Conclusions

The intermediate 2-glycylamino-5-nitrobenzophenone, a colourless substance, was isolated in high yield by hydrolysing nitrazepam in 0.1 M hydrochloric acid. These findings amend earlier reports (Inotsume and Nakono, 1980) that the product cannot be isolated by solvent extraction and that it is yellow in colour (Han et al., 1977).

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References

Davidson, A.G., Chee, S.-M., Millar, F.M. and Watson, D., Isolation and identification of an alkali-catalyzed hydrolysis product of nitrazepam. *Int. J. Pharm.*, 63 (1990) 29–34.

Han, W.W., Yakatan, G.J. and Maness, D.D., Kinetics and mechanisms of hydrolysis of 1,4-benzodiazepines: III Nitrazepam. *J. Pharm. Sci.*, 66 (1977) 795–798.

Inotsume, N. and Nakano, M., Reversible ring-opening reactions of nimetazepam and nitrazepam in acidic media at body temperature. *J. Pharm. Sci.*, 69 (1980) 1331–1334.