Bioorganic & Medicinal Chemistry Letters, Vol.1, No.3, pp. 169-172, 1991
Printed in Great Britain

0960-894X/91 $3.00 + .00

STABILITIES OF TRYPTOPHANYLPHENETHYLAMIDES TO ACID AND ALKALINE CONDITIONS.

D.C. Horwell, G. Ratcliffe and E. Roberts.*
Parke-Davis Research Unit, Addenbrookes Hospital Site, Hills Road, Cambridge CB2 2QB, UK.

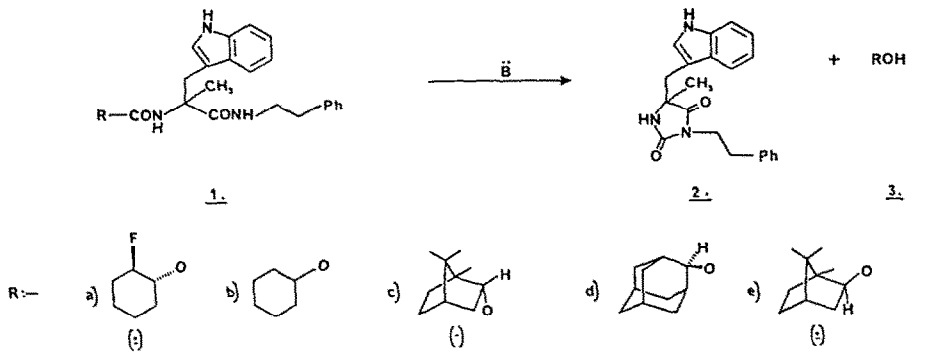
(*Received* 6 *February* 1991)

ABSTRACT : The stability of various alkyloxycarbonyl-α-methyl-tryptophanylphenethylamines in acid media and their decomposition to hydantoins in basic media are discussed.

A potential drug candidate must fulfill a number of criteria if it is to be progressed to clinical evaluation. One such criterion is the possession of a pharmaceutically useful half-life.[1] Such a drug candidate must be resistant to the chemical environment it will encounter by the chosen route of administration. An orally administered drug, for example, must be stable to the acidic pH and digestive enzymes of the stomach and the relative alkalinity of the small intestine. It may then be absorbed through the gastrointestinal (GI) lumen to the blood stream, where further metabolism may occur until diffusion from the blood to the target tissue enables the drug to be bioavailable and exert its desired effect.

We have reported novel 'dipeptoid' compounds 1 based on the C-terminal tetrapeptide of cholecystokinin (CCK-30-33)[2,3] for which the fulfilment of these critera needed to be established, particularly because these 'dipeptoids' each contained a urethane moiety of potential acid and alkaline lability.

FIGURE 1



Indeed, it was found that when compounds of type 1 were subject to strong alkali, rapid cyclization to the hydantoin 2 occured.[4] We therefore devised an NMR method for determining the rates of base cyclization and acidic hydrolysis using ^{1}H NMR spectroscopy with the 'dipeptoids' whose N-terminal blocking group was varied, whilst all other functionalities remained constant. The conditions under which the stability of the compounds were tested are more severe than those found in the GI tract. A protic solvent was required, our choice being aqueous methanol due to the compounds insolubility in water. The pH of the two test conditions were well beyond the extremes of those in vivo, i.e. acid at pH 1 and base at pH 12, whereas the small intestine have max pH ≈ 8.0 and stomach min pH ≈ 2.0.[6]

METHODS

a. Base Stability

Compound 1 (20 μmol) was dissolved in CD_3OD (0.5 cm^3) to which was added NaOD in CD_3OD (0.1 mL of a 0.2M soln, 20 μmol). The NaOD solution was prepared by 50-fold dilution of 40% NaOD in D_2O, available commercially,[5] with CD_3OD. Compound 1 was thereby subjected to a stoichiometric amount of base. The resulting solution was of pH = 12.

b. Acid Stability

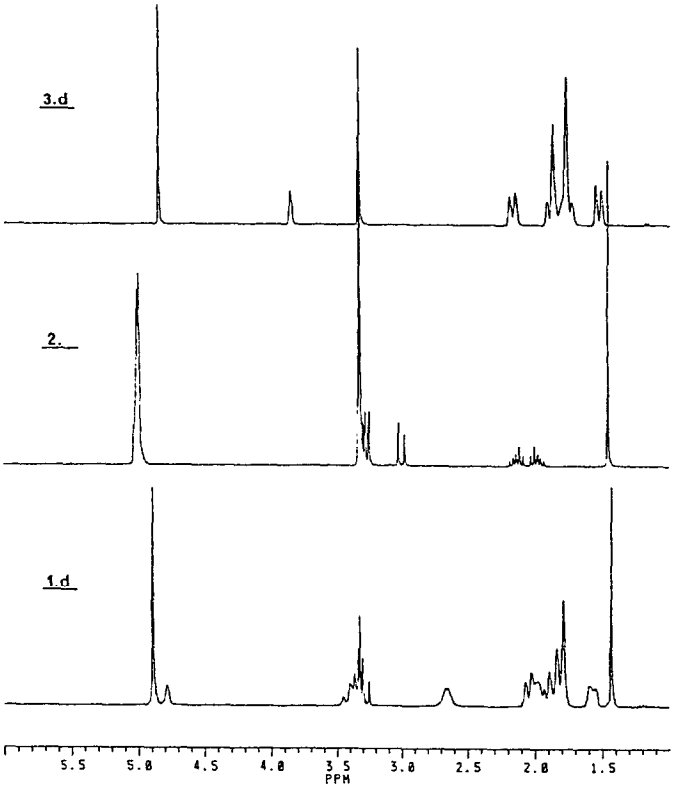
Compound 1 (20 μmoles) was dissolved in DCl in CD_3OD (0.6 mL of a 0.1M, 20 μmol). The acid was prepared by 10-fold dilution of 37% DCl in D_2O, available commercially,[5] with CD_3OD. The resulting solution was of pH = 1.0.

c. ^{1}H NMR

The reactions of 1 to these acid and alkaline conditions were monitored directly by ^{1}H NMR spectroscopy (Bruker AM 300). Spectra were recorded at 300 K, with 16 K data. Signal averaging of 64 transients together with an exponential multiplier (LB = 0.5 Hz) was needed to get adequate signal/noise ratio (S/N) for good integrals. The spectral reference was TMS = 0.0 ppm. Two sets of signals were integrated to monitor the course of the reactions. Those marked (■) in Fig. 2 show the fate of the protecting group indicated by formation of the alcohol, assigned by the methyne singlet δ = 3.85 ppm, whilst those marked (▲) indicate formation of hydantoin. This shows a characteristic doublet at 3.05 ppm in CD_3OD in Fig. 2.

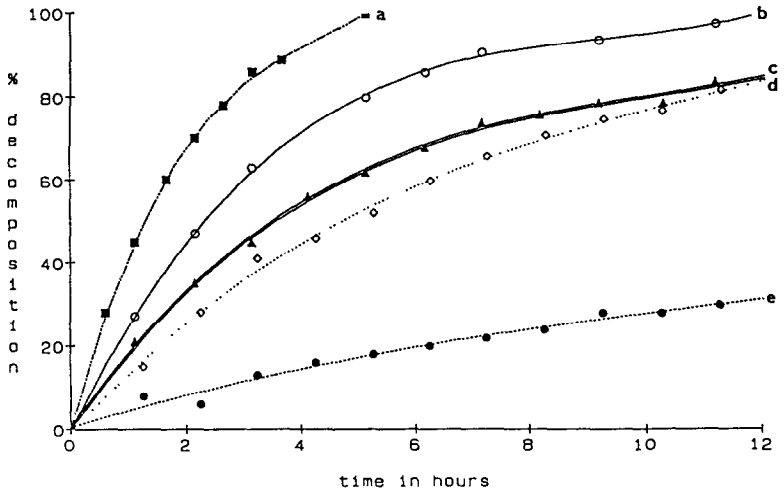
Our preferred N-terminal carbamate groups 1 c,d and e[2,3] show less than 50% decomposition after 4 hours at pH 12. In vivo drugs are not exposed to such

FIGURE 2. ^{1}H NMR CHANGES ON BASE ADDITION.



The graph below shows the results of the base stability experiments.

FIGURE 3. GRAPH SHOWING ALCOHOL LOSS IN BASIC MEDIA.[7]



harsh conditions of pH. Interestingly, the exo-borneol derivative, **1e** cyclized to the hydantoin far more slowly than the endo-borneol, **1c** under the alkaline conditions, whereas **1c** was similar to the 2-adamantanol **1d**. All the compounds **1a – e** tested for acid stability showed no decomposition at pH 1 over a period of 12 hours. These compounds are also stable in neutral organic solutions.

We therefore conclude from the point of view of chemical stability that compounds which contain these 'dipeptoid' carbamates are viable moieties for incorporation into drug candidates.

ACKNOWLEDGEMENTS

The authors gratefully acknowledge the synthetic skills given by M. Higginbottom and K. Martin.

References and Notes

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4a. The hydantoin **2** was identified by spectroscopy and independent total synthesis from reaction of α-methyl tryptophanyl phenethylamide with phosgene. IR (neat) 1769, 1704cm^{-1}; NMR ($CDCl_3$) δ 1.39 (3H, s), 2.48–2.67 (2H, ABq, J 14.5Hz), 3.58 (2H, t, J 8Hz), 5.65 (1H, s), 6.96 (1H, d, J 2Hz), 7.09–7.30 (7H, m), 7.31 (1H, d, J 8Hz), 8.13 (1H, s).

b. Hydantoin formation has also been observed in peptides, e.g. C A Dekker, S P Taylor and H S Fruton; J. Biol. Chem. 1942, 145, 253–265.

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b. M J Dennis in Comprehensive Medicinal Chemistry, Vol.5; Ed. C Hansch, Pergamon Press, 1990, 1–39.

7. Each point represents single measurements of a ratio of integrals and are subject to errors of 5%.