Synthesis of N-Methylbutobarbitone-N-(methyl 2,3, 4-tri-O-acetyl-β-D-glucuronide) under Phase Transfer catalysed condition

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Summary: N-Methylbutobarbitone-N-glucuronic acid (protected) was successfully synthesized by a phase transfer catalysed reaction; it was then characterized by proton NMR and mass spectrometry (EI), for use in investigating the human metabolism of butobarbitone.

Introduction

Phase-II metabolism of most drugs and Xenobiotics that make their way into the human body results in the formation of conjugates with the indogenous substances like glucose and glucuronic acid. The aqueous solubility of these conjugates make them easily excretable through the urine [1].

N-Glucosylation and glucuronidation of barbiturates, sulphonamides and other drugs have been reported [2-16] but the evidence for the precise structure of the proposed conjugates has not been supported by modern analytical techniques like nuclear magnetic resonance spectroscopy and mass spectrometry. Synthesis of such required authentic conjugates in a pure state seems to be the major problem. The present paper described the successful synthesis of the N-glucuronic acid (protected) conjugate of N-methylbutobarbitone by adaptation of a published method [17] of the Dess group. The synthetic product was then characterized by proton NMR and mass spectrometry (EI).

Experimental

N-Methylbutobarbitone-N-(methyl 2, 3,4-tri-O-acetyl-β-D-glucuronide) was synthesized, using the method of Dess et al [17] by dissolving N-methylbutobarbitone (0.42 g, 0.002 mole) and benzyltriethylammonium bromide (0.28 g, 0.001 mole) in aqueous sodium hydroxide (1.25M, 2 ml). The resulting solution was added to a solution of methyl 2,3,4-tri-O-acetyl-α-bromoglu-

curonate (0.41 g, 0.001 mole) in chloroform (5 ml). The mixture was stirred vigorously and heated under reflux (3h). After cooling, water (5 ml) was added. The chloroform layer was seprated and washed twice with aqueous sodium hydroxide (1.25M, 3ml) and dried (sodium sulphate). The solvent was removed, yielding gummy material. Recrystallization from aqueous methanol yielded the product (0.5 g, 50%), m.p. 49-53°. Found: C, 53.3; H, 6.3, N, 5.0. $C_{24}H_{34}N_2O_{12}$ requires: C, 53.1, H, 6.2; N, 5.2%. NMR (proton) recorded at 80 MHz on a Bruker model WP80 SY spectrometer gave the following data: ($CDCl_3$): 0.9 (6H, t); 1.1-1.3 (4H, m); 1.9-2.2 (14H, m); 3.35 (3H, s,); 3.85 (3H, s,); 4.1-4.4 (1H, m); 5.2-5.5 (3H, m); 6.15 (1H, d, J=9Hz). Mass spectrum with electron impact ionization, recorded with a VG-Analytical ZAB-1F, spectrometer gave the following data: m/z (%): 483 (M-COOMe, 68), 423 (483-OAc, 13), 411 (16), 363 (423-OAc, 18), 321 (65), 227 (10), 171 (4), 155 (21), 127 (11), 113 (5), 97 (7), 83 (6), 69 (4), 55 (16), 43 (Ac, 100).

Discussion

The previous methods used for the synthesis of protected glucosides and glucuronides [18-22] have generally resulted in rather low yields of the desired conjugates. The real advantage of the adopted Dess method [17] is, that it involves a phase transfer technique, in which the protected bromosugar is held in the organic phase (chloroform), and in this way is somewhat protected from premature attack by the alkali. The N-methylbutobarbitone is dissolved in a slight excess of aqueous alkali, thus generating the N^--anion; this will then attack, nucleophilically, the bromoglucuronide (protected) when the two reactants are brought into intimate contact by the influence of benzyltriethylammonium bromide (a phase transfer catalyst) at the interface. The reaction product will be liphophilic, and so will be distributed predominantly in the organic phase and will consequently be protected from hydrolytic breakdown by the residual alkali. Thus this simple method was found to give excellent yields of the required protected glucuronide conjugate.

The stereochemistry of the linkage at the glucuronide (protected) would be expected to be β, if the attack by the N-methylbutobarbitone anion on the bromosugar had followed the SN^2 mechanism. That the link was actually , was supported by the NMR (proton) analysis of the anomeric proton, which showed a doublet at 6.15 with J=9Hz; these values are consistent with the expected β-glucuronide linkage [18].

The protected N-glucoside of the same barbiturate was also synthesized [23].

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References

1. G.J.Dutton, Glucuronidation of drugs and other compounds, CRS Press Inc., Florida, (1980).
2. B.K. Tang, W. Kalow and A.A. Grey, *Res.Commun.Chem.Path.Pharmac.*, ***21***, *45 (1978).*
3. M.P.Whyte and A.S. Dekaban, *Drug. Metab. and Disp.*, ***5***, *63 (1977).*
4. D.E. Duggan, J.J. Baldwin, B. H. Arison and R.E. Rhodes, *J.Pharmac.Exp.Ther.*, ***190***, *563 (1974).*

5. J.W. Bridges and R.T. Williams, *Biochem.J.*, **83**, *27, (1962).*
6. J.W. Bridges, M.R. Kibby and R.T. Williams, *Biochem.J.*, **96**, *829 (1965).*
7. J.W. Bridges, S.R. Walker and R.T. Williams, *Biochem.J.*, **111**, *173 (1969).*
8. T.Uno and M. Ueda, *Chem.Pharm.Bull.*, **11**, *709 (1963).*
9. F.F.Kadlubar, J.A. Miller and E.C. Miller, *Cancer Research*, **37**, *805 (1977).*
10. R.G. Smith, G.D. Daves, R.K. Lynn and N. Garber, *Biochem. Mass Spec.*, **4**, *275 (1977).*
11. J.L. Wooley and C.W. Sigel, *Drug. Metab. and Disp.*, **7**, *94 (1979).*
12. R.D.G. Woolfenden, *in "Analytical Profiles of Drug Substances" (Vol. 6) Ed. K. Florey, Academic Press, New York, (1977).*
13. P. Nielsen, *Biochem.J.*, **136**, *1039 (1973).*
14. G.D. Paulson, J.M. Giddings, C.H. Lamoureux, E.R. Mansager and C.B. Struble, *Drug. Metab. and Disp.*, **9**, *142, (1981).*
15. J.E. Matusik, C.J. Barnes, D.R. Newkirk and T.Fazio, *J. Ass. Anal. Chem.*, **65**, *828 (1982).*
16. D.D. Giera, R.F. Abdulla, J.L. Occolowitz, D.E. Dorman, J.L. Mertz and R.F. Siek, *J.Agric.Food.Chem.*, **30**, *260 (1982).*
17. D.Dess. H.P.Kleine, D.V. Weinberg, R.J. Kaufman and R.S. Sidhu, *Synthesis*, **13**, *883 (1981).*
18. B.K. Tang, W. Kalow, and A.A. Grey, *Drug Metab. and Disp.*, **7**, *315 (1979).*
19. M.A.M. Al-Sharifi, *Ph.D. Thesis, London 1982.*
20. R.J. Stubbs, *Ph.D. Thesis, London 1982.*
21. W.H. Soine, V.O. Bhargava and L.K. Garrettson, *Drug.Metab. and Disp.*, **12**, *792 (1984).*
22. B. Berrang, C.E. Twine, G.L. Hennesse and F.I.Carroll, *Synth. Commun.*, **5**, *231 (1975).*
23. B.Ahmad and J.W. Powell, *Unpublished results.*