SYNTHESIS OF 1,1,3,3-($^2H_4$)-4-HYDROXYDEBRISOQUINE SULPHATE

Claus O.Meese

Dr.Margarete Fischer-Bosch-Institut für Klinische Pharmakologie
Auerbachstraße 112, D-7000 Stuttgart 50 (FRG)

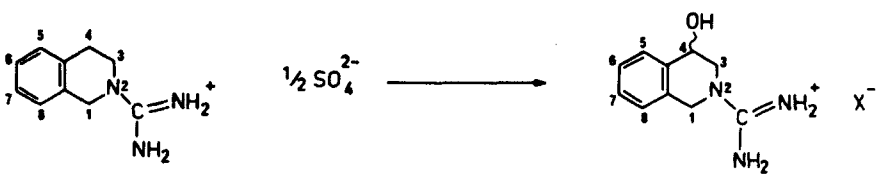
SUMMARY

An efficient synthesis of the analytically useful title compound 2c (1,1,3,3-($^2H_4$)-4-hydroxydebrisoquine sulphate), utilising a two-step deuteration($^1H/^2H$ exchange of 3-nitromethylphthalide, 4, and $LiAl^2H_4$ reduction of 3,3-($^2H_2$)-4-hydroxy-1,2,3,4-tetrahydroisoquinolin-1-one, 6), is described.

Key words: Deuteration, debrisoquine metabolite

INTRODUCTION

Debrisoquine sulphate 1a (1,2,3,4-tetrahydroisoquinoline-2-carboxamidinium sulphate) (1), a potent antihypertensive agent, is also used for the in vitro determination of the 4-hydroxylase activity of microsomal fractions of human liver (2). In order to develop a sensitive and specific gas chromatographic-mass spectrometric assay for 4-hydroxydebrisoquine 2a, the major metabolite of 1a (Scheme 1) in vivo and in vitro (2-4), a deuterated analogue of 2a was required. A previously reported (2) preparation involved the oral administration of the chemically prepared decadeuterated analogue 1b to a human subject who had been phenotyped as a homozygous extensive metaboliser, and the subsequent isolation of the nonadeuterated compound 2b from urine. However, as this tedious approach



1a : $^2H_0$
1b : 1,1,3,3,4,4,5,6,7,8-$^2H_{10}$

2a : $^2H_0$ ($X^- = ½ SO_4^{2-}$)
2b : 1,1,3,3,4,5,6,7,8-$^2H_9$
2c : 1,1,3,3-$^2H_4$; $X^- = ½ SO_4^{2-}$

Scheme 1

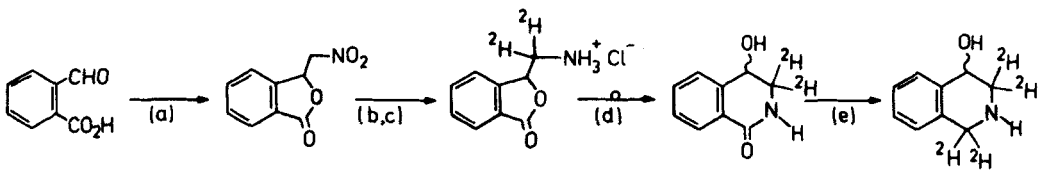
0362-4803/86/040427-06$05.00

Received November 4, 1985
Revised November 25, 1985

yielded only small amounts of the required material, a relatively simple and more effective route to the pure, crystalline tetradeuterated analogue, 2c, was developed.

RESULTS AND DISCUSSION

The method shown in Scheme 2 (5) is based on the ^{1}H-NMR spectrometric observation that partial deuterium exchange of the methylene protons α to the nitro group in 3-nitromethylphthalide 4 occurred in the presence of deuterium oxide and a catalytic amount of anhydrous potassium carbonate (6). Thus, 3-nitromethylphthalide 4(6-9), readily prepared from o-phthalaldehydic acid, was labelled on a preparative scale by means of repetitive $^{1}H/^{2}H$ exchange and subsequently reduced to 5 with deuterium gas in the presence of 20% $^{2}HCl/^{2}H_2O$ and palladium-on-carbon as catalyst (10).



3 4 5 6 7

(a) CH_3NO_2, OH^- (b) $^{2}H_2O, CO_3^{2-}$

(c) $^{2}H_2, ^{2}HCl, Pd/C$ (d) $(C_2H_5)_3N, CH_3OH$

(e) $LiAl^{2}H_4$ (f) $CH_3S\text{-}C(NH_2)_2^{+}$ 1/2 SO_4^{2-}

(f)

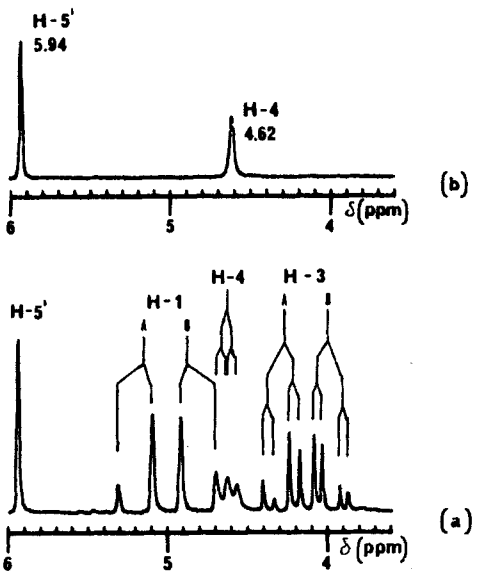
Scheme 2

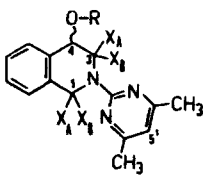
2c

Rearrangement of the free base of 5 followed by reductive deuteration of 6 with lithium aluminium deuteride according to methods described for unlabelled material (11), afforded the crystalline $^{2}H_4$-labelled precursor 7. Final guanidination (1,4,11,12) of the labelled tetrahydroisoquinoline 7 with S-methylisothiouronium sulphate completed the chemical synthesis of the title compound 2c.

In contrast to previous reports, the yields of the individual steps are satisfactory, in addition, all compounds are nicely crystalline and therefore easily purified by recrystallisation. The efficiency of the two-step deuteration is demonstrated by the isotopic composition of the intermediates (see Experimental Section) and of the title compound. Thus, the hemisulphates 2a and 2c were converted by treatment with acetylacetone into

their neutral, crystalline pyrimidine derivatives 8a(13) and 8b. The resultant partial ^{1}H-NMR spectra of 8a and 8b, depicted in Fig.1, demonstrate the large spectral simplification gained when the strongly coupled ring protons H-1 and H-3 are selectively replaced by deuterium. Finally, the 4-(tert.-butyldimethylsiloxy) derivatives 9a and 9b were prepared in order to establish the isotopic composition of 2c quantitatively by mass spectrometry. The deuterium content (5% $^2H_3$,92% $^2H_4$,3% $^2H_5$) and the extremely low blank ($^2H_0$ referred to $^2H_4 \leqslant 0.05\%$) suggest that the analytically pure tetradeuterated metabolite 2c, which is now available by a convenient synthetic procedure, should be suitable for use as an internal standard in a sensitive stable isotope dilution assay.





8a : X = ^{1}H , R = H
8b : X = ^{2}H , R = H
9a : X = ^{1}H , R = Si
9b : X = ^{2}H , R = Si

Figure 1

Partial ^{1}H-NMR spectra (OH decoupled at δ = 1.94 ppm) of 8a (a) and 8b (b), solvent $C_6{}^2H_6$.

EXPERIMENTAL SECTION

TLC: SiO_2 60-F_{254}(E.Merck); solvent systems used: (I) EtOAc/n-hexane(2:3), (II)EtOAc/MeOH/Et_3N(90:10:2), (III) EtOAc/AcOH(98:2).- ^{1}H-NMR:80 MHz, Bruker WP 80, shifts in ppm (δ-scale); int.standard TMS ($CDCl_3$,C_6D_6) or 3-(trimethylsilyl) propanoic acid-$^2H_4$ sodium salt (^2H_2O).- MS(70 eV, PI/EI):Hewlett-Packard 5985, fragments reported as m/z (relative abundance,%).- Melting points (m.p.), uncorrected (Electrothermal).-

3-Nitromethylphthalide, 4, was prepared from o-phthalaldehydic acid, 3, in 40% yield as described (6). The crude product was recrystallised from boiling 2-propanol (3.5 ml/g), m.p.129-130°C (m.p. 129-131°C(6)); TLC:R_f 0.34(I).

3-(Amino-$^2H_2$-methyl)-phthalide hydrochloride, 5

A stirred mixture of dry dioxane (40 ml), anhydrous potassium carbonate (20mg), phthalide 4(8.40g, 43.5 mmol), and ^2H_2O(4ml,0.2mol,Fluka, > 99.8%^2H) was heated under an argon atmosphere in an oil bath(110-140°C). After azeotropic water removal (b.p.87°C) a small sample was withdrawn and poured into 0.1N HCl. The yellow precipitate was dried (P_2O_5) and dissolved in $CDCl_3$. The NMR spectrum showed a singlet for H-3(6.13ppm) and a weak broad signal at about 4.7 ppm, indicative of 5-10 mol% of residual $C\underline{H}_2NO_2$ or $C^1\underline{H}^2HNO_2$. A mixture of deuterium oxide (4ml) and dry dioxane (20ml) was then added again and water was removed azeotropically. After a third $^1H/^2H$ exchange using the same solvent mixture the reaction mixture was concentrated under argon until the boiling temperature reached ca. 92°C. It was then cooled to room temperature with stirring and diluted with 20ml of dry dioxane. Palladium-on-carbon (10%, 5g), 10ml of 20% $^2HCl/^2H_2O$(Fluka, > 99.5% ^2H), and 30 ml of $C_2H_5O^2H$ (E.Merck, 97%, > 99%^2H) were then added and the suspension was vigorously stirred under an atmosphere of deuterium gas (Linde, > 99.5% ^2H) for 11 days (10). The mixture was filtered, and the residue was first washed with ethanol then with water. The combined filtrates were evaporated to dryness, washed with hot toluene, and dried to give 7.85g (90%) of essentially pure, crystalline salt 5. An analytical sample was recrystallised twice from boiling ethanol; m.p. 259-261°C(dec.). Calc. for $C_9{}^1H_8{}^2H_2ClNO_2$ (201.6) C 53.60%,$^1H+^2H$ 6.00%, Cl 17.58%, N 6.95%; found C 53.64%, $^1H+^2H$ 6.19%, Cl 17.56%, N 7.02%.- TLC: R_f 0.27(II).- NMR(^2H_2O): 5.98(s,1H,H-3),7.57-8.09(m,4H,H-5,6,7,8).-

3,3-$^2H_2$-4-Hydroxy-1,2,3,4-tetrahydroisoquinolin-1-one, 6

A mixture of 5(800 mg, 4.ommol), methanol (70ml), and triethylamine (10ml) was heated at 50°C for 48h. The reaction mixture was concentrated in vacuum and the resultant solid residue was treated with 65 ml of hot tetrahydrofuran. After cooling, the suspension was filtered through a short column of silica gel (50g). Collection of the appropriate fractions and evaporation gave 523 mg of crude, crystalline product which was purified by dissolution in 45 ml of boiling EtOAc and precipitation by addition of about 50 ml of n-hexane to yield 439 mg (66%) of lactam 6, m.p.163-164°C. Calc. for $C_9{}^1H_7{}^2H_2NO_2$(165.2) C 65.44%, $^1H+^2H$ 6.71%, N 8.48%; found C 65.23%, $^1H+^2H$ 6.87%, N 8.32%.- TLC: R_f 0.42(II),R_f 0.37(III).- NMR($CDCl_3$) 3.2 (broad s, ~1H,OH), 4.85(s,1H,H-4),6.8(broad s,1H,NH),7.2-7.7(m,3H,H-5,6,7),7.98(d,J=7.3 Hz,1H,H-8).-The isotopic composition was determined from the mass spectrum of the N,O-bis-trifluoroacetyl derivative of 6 (M=357) by comparison with the

mass spectrum of the corresponding unlabelled derivative using the molecular ion: 3% $^2H_1$,94% $^2H_2$,3% $^2H_3$. No rearrangement of 5 to 6 was observed in boiling 2-propanol/Et_3N during 4 h.

1,1,3,3-$^2H_4$-4-Hydroxy-1,2,3,4-tetrahydroisoquinoline, 7

To a stirred suspension of lithium aluminium deuteride (1.05g,25.5mmol, E.Merck, > 90%, > 98%^2H) in 25ml of dry tetrahydrofuran was added 909mg (5.5 mmol) dry lactam 6 under an argon atmosphere. After 5h reflux, the mixture was cooled(0°C) and excess reagent was destroyed by slow addition of 5 ml MeOH followed by 15ml of 30% aqueous NaOH. The mixture was then diluted with diethyl ether (100ml) and filtered. The residue was thoroughly extracted with Et_2O(6x100ml), the combined extracts were washed with about 20% aqueous brine(20ml), dried (Na_2SO_4), and evaporated to give essentially pure base 7 as a light yellow oil (815mg, 97%) which slowly crystallised, m.p.81.5°C (from EtOAc/n-hexane). Calc. for $C_9{}^1H_7{}^2H_4NO$(153.2) C 70.55%, $^1H+^2H$ 9.86%,N 9.14%; found C 70.32%, $^1H+^2H$ 10.08%, N 9.19%.- TLC:R_f 0.11(II).- NMR($CDCl_3$):2.7(broad s,OH,NH), 4.52(s,1H,H-4),6.95-7.5 (m,4H,H-5,6,7,8).-

1,1,3,3-$^2H_4$-4-Hydroxydebrisoquine sulphate, 2c

S-Methylisothiourium sulphate (275mg,0.99mmol) and 7(303mg,1.98mmol) were heated at 40-50°C for 12h in 2 ml of water. The mixture was then refluxed and the precipitate was dissolved by addition of water (2ml). The hot solution was treated with activated carbon and filtered. On cooling (0°C) for 8 h the pure sulphate crystallised (267mg). Evaporation of the filtrate to about 1 ml gave a further crop of crystals. The combined fractions were recrystallised from boiling water (1.5ml/0.1g) to yield 322 mg(66%) of pure 2c,m.p260-262°C(dec.). Calc.for $C_{10}{}^1H_9{}^2H_4N_3O$ x $0.5H_2SO_4$(244.3) C 49.17%, $^1H+^2H$ 7.42%, N 17.28%,S 6.56%; found C 49.09%, $^1H+^2H$ 7.58%,N 17.14%,S 6.75%.-NMR(^2H_2O):5.00(s,1H,H-4), 7.2-7.6(m,4H,H-5,6,7,8).-

2-(4',6'-Dimethyl-2'-pyrimidinyl)-4-hydroxy-1,2,3,4-tetrahydroisoquinoline 8a (M=255.3) and the labelled analogue 8b(M=259.3) were prepared in 68% yield in analogy to previous reports (13,14); m.p.131.5°C and R_f 0.41 (I) were obtained for both compounds.-NMR(C_6D_6,OH decoupled at 1.94 ppm),8a:2.14(s,6H,CH_3), 4.00(dd,J_{AB}=12.9 Hz,$J_{B,CH}$=4.1 Hz,1H,H-3_B), 4.26(dd,J_{AB}=12.9 Hz,$J_{A,CH}$=5.4 Hz,1H,H-3_A),4.62(dd,J=4.1 Hz,5.4 Hz,1H,H-4),4.84 (d,J_{AB}=17.3 Hz,1H, H-1_B),5.17(d,J_{AB}=17.3 Hz,1H,H-1_A),5.94(s,1H,H-5'),6.8-7.5(m,4H,H-5, 6,7,8).-8b:2.14(s,6H,CH_3),4.62(s,1H,H-4), 5.94(s,1H,H-5'), 6.8-7.5(m,4H,H-5,6,7,8).- The iso-

topic composition of 2c was quantitatively determined by mass spectrometry of the O-tert.-butyldimethylsiloxy derivative 9b(M=373) in comparison with unlabelled 9a(M=369) using the abundant $M^{+\cdot}$-57 fragments at m/z 316 (9b) and m/z 312(9a), respectively: 5% $^2H_3$, 92 % $^2H_4$, 3% $^2H_5$; $^2H_0$ (referred to $^2H_4$) $\leqslant$ 0.05%.

ACKNOWLEDGEMENT

Excellent mass spectrometric and technical assistance of Dr.C.Fischer and Mr.B.Borstel, respectively, is gratefully acknowledged. Special thanks are due to Prof.Ramuz (Hoffmann-La Roche, Basel) for a gift of unlabelled reference material.

REFERENCES AND NOTES

1. Wenner W.-J.Med.Chem. 8: 125 (1965)
2. Kahn G.C., Boobis A.R., Murray S., Brodie M.J. and Davies D.S.- Br.J.clin.Pharmac. 13: 637 (1982)
3. Murray S., Kahn G.C., Boobis A.R. and Davies D.S.-Int.J.Mass Spectrom. Ion Phys. 48: 89 (1983)
4. Idle J.R., Mahgoub A., Angelo M.M., Dring L.G., Lancaster R. and Smith R.L.- Br.J.clin.Pharmac. 7: 257 (1979)
5. All synthetic steps described for deuterated compounds were carried out with unlabelled material as well .
6. Whelton B.D.and Huitric A.C.- J.Org.Chem. 35: 3143 (1970)
7. Baer H.H. and Achmatowicz B.- J.Org.Chem. 29: 3180 (1964)
8. Ullyot G.E., Stehle J.J., Zirkle C.L., Shriner R.L. and Wolf F.J.- J.Org.Chem. 10: 429 (1945)
9. Dey B.B. and Srinivasan T.K.- Arch.Pharm.Ber.Dtsch.Pharm.Ges. 275: 397 (1937)
10. Owing to a considerable primary kinetic isotope effect, catalytic reduction of the nitro group of 4 with molecular deuterium proceeds about ten times more slowly than reduction with hydrogen (5). Nevertheless, the reaction conditions described avoid any possible catalytic $^2H/^1H$ exchange.
11. Ram S., Saxena A.K. and Jain P.C.- Ind.J.Chem. 16B: 1019 (1978)
12. Malcolm S.L. and Marten T.R.- Anal.Chem. 48: 807 (1976)
13. Lennard M.S., Silar J.H., Smith A.J. and Tucker G.T.- J.Chromatogr. 133: 161 (1977)
14. Murray S. and Waddell K.A.- Biomed.Mass Spectrom. 9: 466 (1982)