SYNTHESIS OF SINGLY ^{2}H-, ^{3}H-, AND ^{14}C- AND DOUBLY LABELED ACETAMINOPHEN, PHENACETIN, AND p-ACETANISIDINE

Kenneth K. Chan*
School of Pharmacy, University of Southern California,
Los Angeles, California 90033 and
K. Sandy Pang
Department of Pharmaceutics, College of Pharmacy,
University of Houston, Texas 77030

SUMMARY

Several efficient procedures for the synthesis of deuterium, tritium, and ^{14}C-labeled acetaminophen, phenacetin, and p-acetanisidine are described. p-Aminophenol was acylated by the appropriate acetic anhydride under mild conditions yielding labeled acetaminophen. With O-alkylation using $NaCH_2SOCH_3$ and appropriate labeled and unlabeled alkyl halides, labeled phenacetin and p-acetanisidine were also obtained. Phenacetin labeled both with ^{14}C on the acyl group and deuterium on the ethoxy group was synthesized in high yield by acylation of p-phenetidine-d_5. The last compound was obtained by acid hydrolysis of phenacetin-d_5 synthesized previously.

Key Words: ^{3}H-Acetaminophen, ^{2}H-Acetaminophen, ^{2}H-Phenacetin, ^{2}H-p-Acetanisidine, ^{14}C-^{2}H-Phenacetin

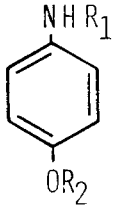
INTRODUCTION

Of the p-aminophenol, antipyretic and analgesic drug class, acetaminophen, (1, N-acetyl-p-aminophenol), and phenacetin (3, p-ethoxyacetanilide) are the most widely used over-the-counter drugs and in pediatrics. The p-methoxy analog, p-acetanisidine (2) has not been used as such, however. Because of the high incidence of renal and hematologic toxicities (1,2), phenacetin has been removed from most of the analgesic formulations. Nonetheless, toxicity associated with an overdose of acetaminophen is still a cause for concern (3) and has been linked to its metabolic activation which purports to yield the highly reactive

* To whom correspondence should be addressed.

0362-4803/82/030321-09$01.00

Received February 13, 1981
Revised July 22, 1981



1 :	R_1= -C(=O)CH_3, R_2=H,	acetaminophen
1a:	R_1=-C(=O)C^2H_3, R_2=H,	acetaminophen-d_3
2 :	R_1= -C(=O)CH_3, R_2= CH_3,	p-acetanisidine
2a:	R_1= -C(=O)C^2H_3, R_2= CH_3,	p-acetanisidine-d_3
2b:	R_1= -C(=O)C^2H_3, R_2= C^2H_3,	p-acetanisidine-d_6
2c:	R_1= -C(=O)CH_3, R_2= C^2H_3,	p-acetanisidine-d_3
3 :	R_1= -C(=O)CH_3, R_2= C_2H_5,	phenacetin
3a:	R_1= -C(=O)C^2H_3, R_2= C_2H_5,	phenacetin-d_3
3b:	R_1= -C(=O)CH_3, R_2= $C_2{}^2H_5$,	phenacetin-d_5
3c:	R_1= -C(=O)C^2H_3, R_2= $C_2{}^2H_5$,	phenacetin-d_8
3d:	R_1= -C(=O)C^3H_3, R_2= C_2H_5,	phenacetin-^3H
3e:	R_1=-^{14}C(=O)CH_3, R_2= $C_2{}^2H_5$,	^{14}C-phenacetin-d_5
4 :	R_1=H, R_2= C_2H_5,	p-phenetidine
4a:	R_1=H, R_2= $C_2{}^2H_5$,	p-phenetidine-d_5

acetamidoquinone species (4). Many of the metabolic studies requires the use of analogs labeled with stable and radioactive isotopes (5-7). Recent investigations of the kinetics of metabolism of acetaminophen and phenacetin (8-10) require the use of minute amounts of these compounds with very high specific activity to ensure linearity of the kinetic processes. Although several methods have been reported for the synthesis of these compounds labeled with different isotopes at various positions, some of these methods require rather drastic conditions and extensive clean-up procedures and are not readily adopted to very small scale. We now report several alternate micromethods for the labeling of phenacetin, p-phenetidine, p-acetanisidine, and acetaminophen, including one where both stable and radioactive isotopes are incorporated at different positions of the same molecule.

EXPERIMENTAL

Mass spectra and selected ion measurement were performed by a quadrupole gas chromatograph-mass spectrometer-data system (GC-MS-DS, HP-5985A, Hewlett Packard, Palo Alto, California). All chemical ionization mass spectra were obtained using methane as the reagent gas. Percentage of stable isotope

labeling was assessed either by direct calculation from the relative abundance of ions or by the selected ion monitoring, all performed under chemical ionization mode. The calculation was performed according to the following equation:

$$\frac{(M_i)_{\text{labeled,corrected}}}{\sum_{i=M-x}^{M+1} (M_i)_{\text{labeled,corrected}}}$$

where M_i = molecular ion + 1(M+1) corresponding to the labeled compound in question under chemical ionization condition, and

x = the number of isotope in the labeled compound.

All molecular ions used have been corrected for the contribution of ions from natural abundance by the parallel comparison of the spectra of the unlabeled compound obtained under identical condition. Radioactivity was measured by a Beckman Liquid Scintillation Counter (Model LS 7500, Irvine, Calif.).

Acetaminophen-d_3 (N-Acetyl-d_3-p-aminophenol, 1a)

To a 20 ml aqueous solution of 2.0 g (13.84 mmole) of recrystallized p-aminophenol hydrochloride (Eastman Kodak, Rochester, N.Y.) and 3 g sodium acetate in a round bottom flask immersed in an ice bath, was added dropwise 1.67 g (15.4 mmole) acetic-d_3 anhydride (99 atom % D, Merck, Rahway, N.J.). After stirring (magnet) in an ice bath for 1 hr, the mixture was brought to room temperature and allowed to stir for an additional 3 hr. The aqueous aolution, which contained floating white crystalline products, was saturated with NaCl and extracted with 3 x 50 ml of ethyl acetate. The combined organic layers were then back extracted once with 20 ml saturated $NaHCO_3$ solution followed by 20 ml H_2O. The ethyl acetate solution was then dried with anhydrous Na_2SO_4 overnight. Evaporation of the solution *in vacuo* afforded 1.7 g of N-acetyl-d_3-p-aminophenol. TLC (silica gel, $CHCl_3$) of the products indicated no starting material remained when examined under UV light. Recrystallization in MeOH:ether (1:6) yielded 1.4 g (70%) of pure crystalline products, mp (uncorrected) 168-170°C (lit.[11] 169-171°C). Chemical ionization (CH_4) mass spectrum (CIMS) of this labeled material and of an authentic acetaminophen gave major fragments at m/z 183/180 ($M+C_2H_5^+$) and 155/152 (MH^+). Selected ion monitor mass spectrometry using

chemical ionization mode (m/z 155/152) indicated 99% deuterium labeling consistent with that of the starting material. Repeating the synthesis gave a purified product of 83% yield.

Phenacetin-d_3, -d_5, and -d_8 (N-Acetyl-d_3-p-ethoxyaniline, 3a, N-Acetyl-p-ethoxy-d_5-aniline, 3b, and N-Acetyl-d_3-p-ethoxy-d_5-aniline, 3c, respectively) and p-Acetanisidine-d_3, -d_6, and -d_3 (N-Acetyl-d_3-p-methoxyaniline, 2a, N-Acetyl-d_3-p-methoxy-d_3-aniline, 2b, and N-Acetyl-p-methoxy-d_3-aniline, 2c, respectively)

Procedure A. NaH, 5.0 g, (50% oil suspension, Ventron, Beverly, Mass.) was placed in a three-neck round bottom flask and was washed with 10 x 15 ml dry petroleum ether. Each washing was emptied by decantation. The remaining trace of the solvent was removed by a stream of N_2. While maintaining under N_2 atmosphere, the flask was then immersed into an ice bath. Dimethylsulfoxide, 50 ml, was added dropwise to the NaH with stirring (magnet). The mixture was then brought to room temperature and was allowed to stir for an additional 2 hr. Acetaminophen-d_3, 0.5 g, was dissolved in 2.0 ml of dimethylsulfoxide in an ampoule enclosed with a rubber serum cap and the atmosphere replaced by N_2. Ethyl-d_5 iodide, 0.75 g, (Merck, Rahway, N.J.) was added into the vial through the rubber seal via a syringe. A 5 ml empty syringe was inserted into the rubber seal and another 5 ml syringe containing 2.0 ml $NaCH_2SOCH_3$ solution was then inserted. While stirring, the sodium methylsulfinylmethide solution was added dropwise to the vial and the addition lasted for approximately 1.75 hr. At about 1 hr later, the reaction mixture was poured and the vial rinsed into 100 ml ice cold 0.1 N HCl. White crystalline precipitate rapidly appeared and was collected by suction filtration to afford 0.45 g (75%) crude phenacetin-d_8. For smaller scale reaction, it was necessary to extract the filtrate with ethyl acetate to obtain additional amount of products. TLC (silica gel, $CHCl_3$, UV light) showed a single spot having the same R_f value (.79) as that of the authentic unlabeled phenacetin. Repeating this synthesis with another 0.5 g of acetaminophen-d_3 yielded 0.37 g (61%) of 3c. These two batches were combined and, after recrystallization in MeOH-ether (1:6), 0.62 g of pure phenacetin-d_8 was obtained, mp (uncorrected) 131-133°C (lit. [12] 134-135°C). CIMS gave major

fragments parallel to those of the undeuterated authentic sample, d_8/d_0, m/z 216/208 ($M+C_2H_5^+$) and 188/180 (MH^+). Selected ion monitoring (m/z 188/187) using chemical ionization mode indicated 65.5% d_8, 26.9% d_7, and 8.0% d_6. Similarly, 2a, 2b, 2c, 3a, and 3b were synthesized using 0.1 g of the appropriately labeled and unlabeled acetaminophens and alkyl halides. The respective yields and identification data were: 2a, 71% (0.08 g), mp (uncorrected) 125-126°C (lit. [11] 130-132°C), CIMS, d_3/d_0 at m/z 169/166 indicating 60.8% d_3; 2b, 62% (0.07 g), mp (uncorrected) 125-126°C (lit. [11] 130-132°C), CIMS d_6/d_0 at m/z 172/166 indicating 52.8% d_6; 2c, 80% (0.09 g), mp (uncorrected) 124-126°C (lit. [11] 130-132°C), CIMS, d_3/d_0 at m/z 169/166 indicating 98% d_3; 3a, 65% (0.08 g), mp (uncorrected) 132-133°C (lit. [12] 134-135°C), CIMS, d_3/d_0 at m/z 183/180 indicating 62% d_3; 5b, 73% (0.09 g), mp (uncorrected) 132-133°C (lit. [12] 134-135°C), CIMS, d_5/d_0 at m/z 185/182 indicating 98% d_5.

Procedure B. Alternatively, compounds 2a, 2b, 3a, 3b and 3c were synthesized by refluxing the appropriately labeled and unlabeled acetaminophens and alkyl iodides in dry acetone in the presence of anhydrous K_2CO_3 using the method of Allen and Gates (5,13). The yields and identification data were: 2a, 80% (0.88 g), mp (uncorrected) 125-126°C (lit. [11] 130-132°C), CIMS, d_3/d_0 at m/z 197/194 ($M+C_2H_5^+$) and 169/166 (MH^+) indicating 98% d_3 2b, 85% (0.94 g), mp (uncorrected) 125-126°C (lit. [11] 130-132°C), CIMS, d_6/d_0 at m/z 200/194 ($M+C_2H_5^+$) and 172/166 (MH^+) indicating 95% d_6; 3b, 80% (0.98 g), mp (uncorrected) 131-133°C (lit. [12] 134-135°C), CIMS, d_5/d_0 at m/z 185/180 indicating 98% d_5; 3c, 55% (0.26 g), mp (uncorrected) 132-134°C (lit. [12] 134-135°C), CIMS, d_8/d_0 at m/z indicating 188/180, 98% d_8.

p-Phenetidine-d_5 (p-Ethoxy-d_5-aniline, 4a)

Phenacetin-d_5, 98 atom % 0,3 g, was refluxed in 2N HCl in 1:1 H_2O-MeOH for 4 hr. After cooling to room temperature, the light violet mixture was made basic with 5N NaOH and extracted with 2 x 50 ml ethyl acetate. Then the organic solution was extracted with 3 x 10 ml 1N HCl. The aqueous solution was again made basic with 5N NaOH and was extracted with 3 x 25 ml ethyl acetate. After washing with 10 ml H_2O, the ethyl acetate solution was dried over anhydrous

Na_2SO_4 for 2 hr and the organic layer was then filtered. Upon evaporation, it yielded 0.18 g (80%) light brown liquid. CIMS showed major ion at m/z 143 (MH^+) as compared to m/z 138 for the unlabeled p-phenetidine (2). Selected ion monitor (m/z 142/141) revealed 98% d_5 which indicated no back exchange during hydrolysis.

(N-Acetyl-^{3}H-p-ethoxyaniline, 3d)

The bottom portion of a long break-seal ampoule containing 0.0204 g of acetic-^{3}H anhydride (500 mCi/mmole, Amersham Nuclear) was immersed in a Dry Ice-acetone bath, while the remaining portion of the ampoule was heated by a hot air blower. After several minutes of condensation, the seal was broken with a spatula and 0.027 g (0.196 mmole) of p-phenetidine in 1 ml of 0.2N HCl was introduced through the seal into the ampoule via a syringe, followed by the addition of 0.050 g sodium acetate in 1 ml of water. The ground glass joint at the top of the ampoule was enclosed a stopper. The ampoule was removed from the Dry Ice-acetone bath and was vortexed rigorously for 0.25 hr. Then the tube was allowed to stand at room temperature for an additional 1 hr with intermittent vortexing. About 1.5 ml of ethyl acetate was added through the seal and the tube was again vortexed rigorously. After separation, the ethyl acetate layer was carefully removed and placed into extraction tube. The extraction with the remaining aqueous layer was repeated 5 times with 1.5 ml EtOAc each time and the combined ethyl acetate layer was extracted once with 2 ml of 0.2N HCl followed by 2 nl of 0.3N NaOH. Then the organic extract was again washed once with 2 ml H_2O and dried over anhydrous Na_2SO_4 for 2 hr. The ethyl acetate solution and its washings were then transferred, a portion at a time, to a pre-weighed vial and was evaporated under a stream of N_2. The lightly tinted white flakes weighed 0.0302 g (85%). TLC (silica gel, $CHCl_3$, UV light) indicated a single spot with the R_f value (.79) identical to that of phenacetin. Radioactive purity as assayed by TLC (silica gel, ether) and liquid scintillation counting was 94% and specific activity assayed was 563 mCi/mmole.

Phenacetin-^{14}C, $^2H_5$ (N-Acetyl-1-^{14}C-p-ethoxy-d_5-aniline, 3e)

The titled compound was synthesized by a similar procedure using 0.0070 g

(0.049 mmole) acetic-1-^{14}C anhydride (21.4 mCi/mmole) and 0.010 g (0.0845 mmole). The product, after acid-base purification as above, gave lightly tinted white flakes of 0.0123 g (99%). TLC (silica gel, $CHCl_3$ UV light) indicated a single spot with a R_f value (.79) identical to that of phenacetin. Radiopurity as assayed by TLC (silica gel, ether) and liquid scintillation counting was 94% and specific activity 21.3 mCi/mmole. The mass spectrum of a non-radiolabeled sample obtained by a parallel reaction under the identical conditions gave major fragments parallel to those of the authentic unlabeled phenacetin. Selected ion monitor under chemical ionization condition (m/z 185/184) indicated 98% d_5 and no back exchange.

DISCUSSION

Acylation of p-aminophenol by rigorous conditions such as the use of acyl chloride leads to O and N acylation and di-acylation. Using ^{14}C-acetic anhydride at elevated temperatures, Stavchansky and Wu (6) obtained ^{14}C-acetaminophen at high yield and no other acylated product was observed. Using a similar acylating agent with sodium acetate under milder conditions, we have obtained comparable yields. This procedure, however, is simpler, and eliminates the refluxing step. In addition, this procedure can be operated at extremely small scales with good yields and can be generally used in the acylation of thermally labile molecule and/or in the presence of free OH groups. The sodium acetate added as a buffer was not incorporated appreciably (< 3%) into the products since no significant isotope dilution was observed within experimental error.

O-Alkylation of phenol can be accomplished by several methods but only a few are applicable to the labeling synthesis because of the limitation of readily available labeled alkylating agents. O-Methylation using strong base in aprotic polar solvent such as dimethysulfoxide used here has been widely employed in the permethylation of peptides (14) and nucleosides (15). This procedure has been successfully adopted in the labeling of phenacetin and p-acetanisidine using acetaminophen and the corresponding labeled alkyl halides. The procedure is rapid and efficient and can be used conveniently in a very small scale. Although the carbanion reagent is usually prepared using slightly elevated temperature

and allowed to react for several hours (14,15), we found that best results for these syntheses were obtained under our conditions. However, as shown by the selected ion monitor, substantial amount of loss of deuterium (25-40%) resulted in the case of phenacetin and p-acetanisidine labeled with deuterium on the acyl side chanin obtained by this procedure. However, no deuterium loss was observed when only the alkyl halides were labeled. This indicates that fast exchange of isotope occurs at the acyl side chain but not at the alkyl group. This is not unexpected in view of the rather strongly basic condition for the alkylation. Since no isotope exchange was observed in the O-alkyl group, this procedure is still useful in the O-alkylation.

The O-alkylation of Allen and Gates (13) has also been found to be simple and efficient and no loss of label was observed at the acyl side chain and at the alkyl group of labeled phenacetin and p-acetanisidine.

The normal sequence of acylation of p-aminophenol followed by O-alkylation by either of these procedures does not appear to be the best method for the synthesis of doubly labeled phenacetin and p-acetanisidine where the acyl group is labeled with radioactive isotopes and the O-alkyl group labeled with deuterium. Since in this case the limiting reagent is the radiolabeled acetic anhydride, the expected yield will not be very high after extensive purification from the two steps. Additionally, the procedure by Allen and Gates is difficult to be adopted in an ultra-micro scale, and O-alkylation by the dimethylsulfoxide carbanion will lead to substantial exchange of ^{3}H on the acyl side chain. Therefore, phenacetin-d_5 was first synthesized, followed by acid cleavage of the acyl group under acidic condition. No exchange of deuterium occurred at the O-ethyl side chain during the hydrolysis. Thus using purified p-phenetidine-d_5, ^{14}C-phenacetin-d was obtained by acylation with ^{14}C-acetic anhydride in good yield. Similar procedures can be used for the labeling of phenacetin with tritium and deuterium or of the p-acetanisidine analogs. Additionally, since only the $-NH_2$ group is acylated, the use of labeled acyl chloride may be more economical and will avoid the wasting of 50% of labeled material as was in the case of the use of labeled acetic anhydride.

ACKNOWLEDGEMENT:

This work is supported by a PHS Grant GM-27323 of the National Institute of Health (KSP).

REFERENCES

1. Abel, J.A. Clin. Pharmacol. Ther. 12: 583(1970).
2. Beutler, E. Pharmacol. Rev. 21: 73(1969).
3. Jollow, D.J., Thorgeirsson, S.S., Potter, W.Z., Hasimoto, M., and Mitchell, J.R. Pharmacology 12: 251(1974).
4. Jollow, D.J., Mitchell, J.R., Potter, W.Z., Davis, D.C., Gillette, J.R., and Brodie, B.B. J. Pharmacol. Exp. Ther. 187: 195(1973).
5. Garland, W.A., Hsiao, K.C., Pantuck, E.J., and Conney, A.H. J. Pharm. Sci. 66: 340(1977).
6. Stavchansky, S. and Wu, P. J. Labelled Compound Radiopharm. XIV: 337(1978).
7. Freed, C.R. and Murphy, R.C. J. Labelled Compound Radiopharm. XV: 637 (1978).
8. Pang, K.S. and Gillette, J.R. J. Pharmacol. Exp. Ther. 207: 178(1978).
9. Pang, K.S. and Gillette, J.R. J. Pharmacokin. Biopharm. 7: 275(1979).
10. Pang, K.S. and Gillette, J.R. Drug Metab. Disp. 8: 39(1980).
11. The Merck Index, 8th Ed. Merck and Co., Inc. Rahway, N.J., 1968, p.5.
12. ibid. p.8.
13. Allen, C.F. and Gates, J.W. Org. Syn. Coll. 3: 140(1955).
14. Ling, N., Burgus, R., Rivier, J., Vale, S., and Brazean, P. Biochem. Biophys. Res. Comm. 50: 127(1973).
15. De Leenheer, A.P. and Gelig'keus, C.F. Anal. Chem. 48: 2203(1976).