THE SYNTHESIS OF ACETAMINOPHEN-d_4

Curt R. Freed and Robert C. Murphy
Departments of Medicine and Pharmacology,
University of Colorado Medical Center, 4200 East Ninth Avenue,
Denver, Colorado 80262
Received December 23, 1977
Revised April 25, 1978

SUMMARY

Acetaminophen-d_4 (4'-hydroxyacetanilide-2',3',5',6'-d_4) has been synthesized. p-Aminophenol underwent proton exchange in 4N DCl at 150° for one week. This compound was acetylated in acetic anhydride to diacetyl-p-aminophenol and selectively hydrolyzed in 0.1 N HCl to acetaminophen-d_4.

Key Words: Acetaminophen, Deuterium

INTRODUCTION

Acetaminophen (4'-hydroxyacetanilide) is a commonly used over-the-counter analgesic which has been recognized to cause hepatic necrosis in overdose in man and experimental animals (1). Hepatic cytochrome P-450 appears to produce a highly reactive oxidized metabolite which can be trapped by glutathione. In overdose, a toxic acetaminophen metabolite exhausts glutathione stores and covalently binds to the hepatocyte macromolecules; however, the precise nature of this toxic metabolite has not been determined. A benzene ring-deuterated acetaminophen could be useful both for studying possible isotope effects on this hepatotoxicity and for attempting to isolate the covalently bonded toxic metabolite.

Tetradeutero (2,3,5,6-d_4)-acetaminophen (acetaminophen-d_4) has been synthesized from nitrobenzene-d_5 (2) although details of synthesis were not given.

0362-4803/78/0015-0637$01.00

The present paper gives an alternate synthetic route with native p-aminophenol hydrochloride as the starting material.

p-Aminophenol underwent proton exchange in deuterium chloride at elevated temperatures. Acetaminophen itself could not be used for this exchange because the acetyl group was hydrolyzed under the acidic exchange conditions. On cooling the acid exchange reaction mixture, the p-aminophenol crystalized as the hydrochloride salt. This salt was then acetylated by acetic anhydride which produced diacetylaminophenol as characterized by mass spectrometry and IR spectroscopy. Diacetylaminophenol was selectively hydrolyzed to the desired product by heating in 0.1N HCl. The reaction mixture was dried by lyophilization to a white solid and the final product, acetaminophen-d_4, was crystalized from ethanol and water.

EXPERIMENTAL

1. p-Aminophenol-2,3,5,6-d_4

   6.0 grams p-aminophenol·HCl was mixed with 20 ml 20% DCl (Aldrich) and 10 ml D_2O at 150° under nitrogen for three days. Mass spectrum of the reaction mixture using a trimethylsilyl derivative showed 40% d_4, 42% d_3, 15% d_2, 2% d_1, and 0% d_0. This sample was dried by lyophilization and the system recharged with 20 ml 20% DCl and 10 ml D_2O and heating was continued for three additional days at 150°. The reaction mixture was cooled and transparent rod-like crystals were formed with a weight of 5.5 grams for 92% yield. The mass spectrum of the trimethylsilyl (TMS) derivative showed 83% d_4, 16% d_3, 1% d_2, 0% d_1, and 0% d_0. Anal. calculated for $C_6H_3D_4NO$: C, 48.2; ($^1H+^2H$), 5.33; N, 9.37; O, 10.71. Found: C, 47.07; ($^1H+^2H$), 5.61; N, 9.00; O, 10.70 (deuterium measured as hydrogen incorporated into water). The material sublimated at 275-277°C.

2. Diacetyl-p-aminophenol-d_4

   5.0 grams of p-aminophenol·HCl-d_4 was reacted with 30 ml acetic

anhydride and 15 ml acetic acid for three hours at 80°. This mixture was then lyophilized and the white product dissolved in 40 ml H_2O and 40 ml ethanol with stirring at 70°. White flake-like crystals appeared on cooling with recovery of 5.1 grams for an 81% yield. Melting point 152-154°. Anal. calculated for $C_{10}H_7D_4NO_3$: C, 60.95; ($^1H+^2H$), 5.85; N, 7.11; O, 24.36. Found: C, 61.28; ($^1H+^2H$), 5.97; N, 6.88; O, 24.60. IR (KBr): 1750 (ester C = O), 1685 (amide C = O) and 1545 cm^{-1} (amide II).

3. Acetaminophen-d_4

4.5 grams of diacetyl-p-aminophenol-d_4 was dissolved with stirring in 30 ml of 0.1 N HCl and 10 ml of ethanol at 80° under nitrogen. Deacetylation reached its maximum in three hours as determined by gas chromatography using a trimethylsilyl derivative. The reaction mixture was dried with lyophilization to a white solid. This solid was redissolved in 15 ml H_2O and 5 ml ethanol with heating under nitrogen. Coarse, diamond-shaped white crystals appeared on cooling giving 2.5 grams (70%) of the deuterated acetaminophen: M.P. 169-171° (lit[4] 169-170.5). Mass spectrometry showed no back exchange of deuterium had occurred (see Figure 1). Anal. calculated for $C_8H_5D_4NO_2$: C, 62.00; ($^1H+^2H$), 6.21; N, 9.04; O, 20.65. Found: C, 62.04; ($^1H+^2H$), 6.16; N, 8.76; O, 21.02. IR (KBr): 1655 (amide I, C = O) and 1565 (amide II, C = O).

DISCUSSION

The mass spectrum of the trimethylsilyl derivative of acetaminophen-d_4 is presented in Figure 1. The isotopic variants of acetaminophen permitted interpretation of the electron impact induced fragmentation. The molecular ion at m/e 299 is consistent with attachment of two trimethylsilyl moieties and four deuterium atoms to the parent structure. Simple cleavages lead to the loss of CH_3 and ·OTMS radicals at m/e 284 and 210, respectively. The

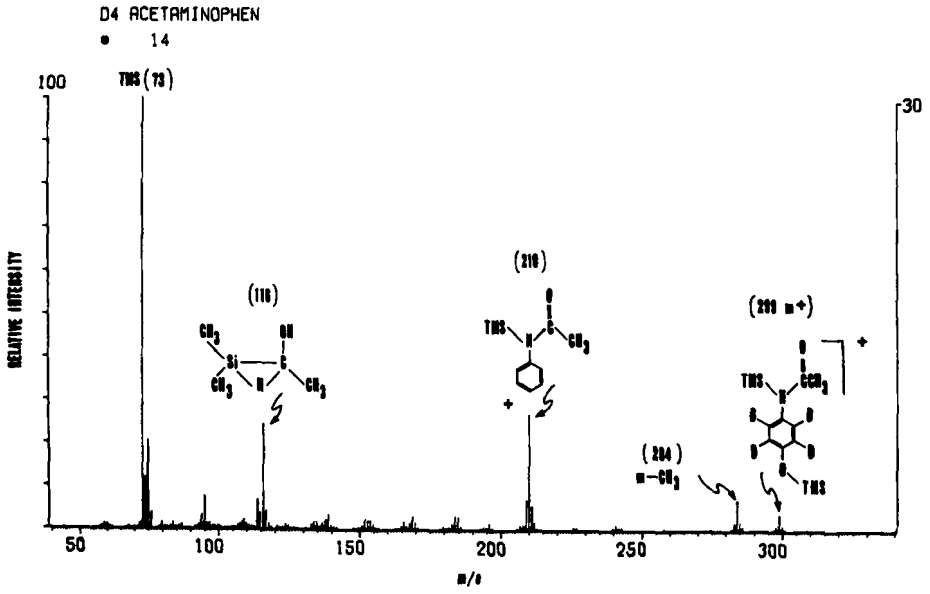
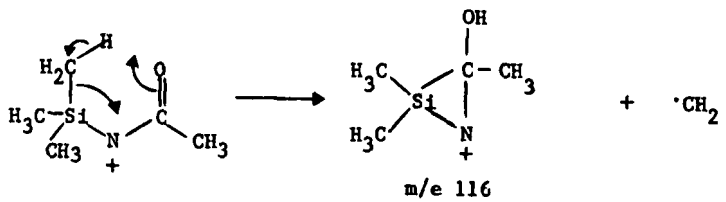


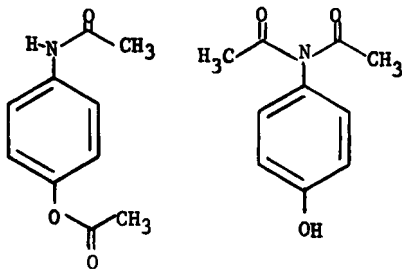
Figure 1: Electron-impact mass spectrum (70eV) of bis (trimethylsilyl) acetaminophen-d_4.

ion at m/e 116 is a rearrangement ion and retains only 2 methyl groups plus one hydrogen of a trimethylsilyl moiety as revealed by derivatizing with TMS-d_9 groups which shift the m/e 116 ion to m/e 123. A possible rearrangement leading to this ion would be cleavage of the nitrogen-aromatic carbon bond with charge retention on the nitrogen. This could then lose methylene initiated by an attack of the carbonyl oxygen on a TMS hydrogen (Scheme I).



SCHEME I

The structural assignment of the diacetylaminophenol was made only after consideration of the mass spectra of the native, d_4, TMS-d_9, and pentafluoropropionyl (PFP) compounds. The aromatic amide of acetaminophen is sufficiently reactive to allow further acetylation and trimethylsilylation. Aliphatic amides such as peptides normally are relatively unreactive to such derivatization. While it may seem obvious that the N-acetyl-p-acetyl compound (A) was formed, physical data available could not rule out N,N-diacetyl-p-aminophenol (B).



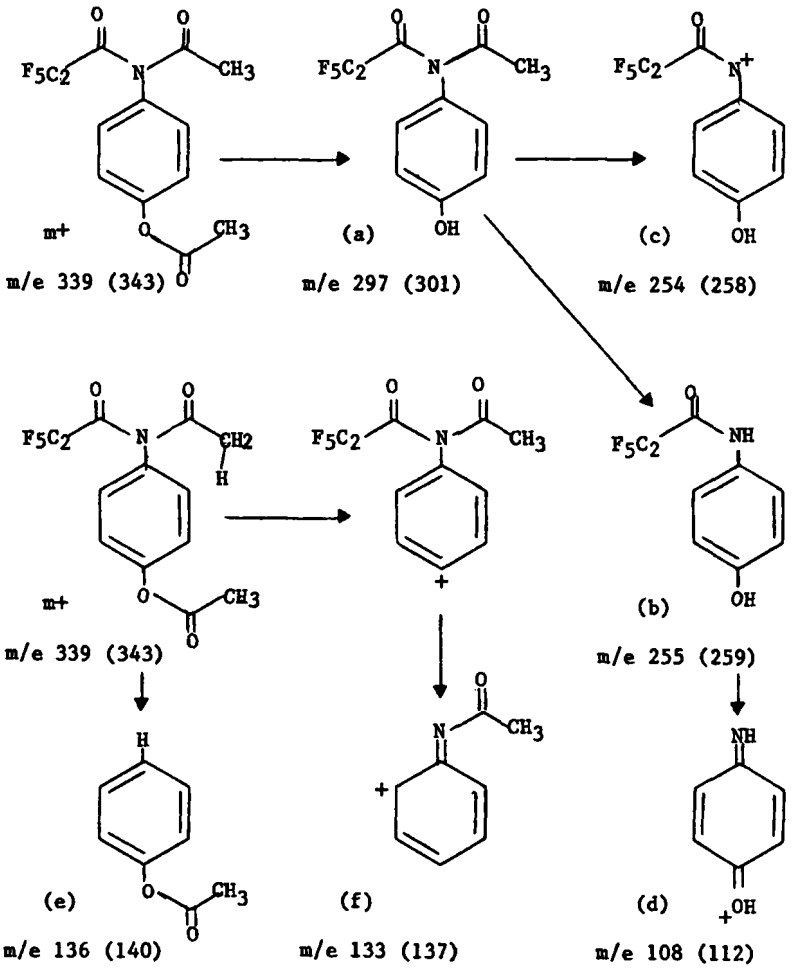
A B

For example, the carbonyl stretch at 1750 and 1695 cm^{-1} could be consistent with the ester-amide of A or the N,N-diacetyl compound B (3). Table 1 presents the mass spectra of the PFP derivative of native diacetylaminophenol-d_4 to support the postulated fragmentation pathways of Scheme II. Sequential loss of ketene (42 mass units) accounts for the high mass region ions at m/e 297 (a) and 255 (b). The ion at m/e 255 can further decompose to the imino-quinone type stabilized ion at m/e 108 (d).

Table 1. Selected ions from the mass spectrum of pentafluoropropionyl-diacetyl-aminophenol.

Ion[1]	Int[2]	m/e d_0	m/e d_4
M+	1.0	339	343
a	5.0	297	301
b	43.1	255	259
c	9.8	254	258
d	85.2	108	112
e	47.1	136	140
f	58.7	133	137
base peak	100.0	43	43

[1] Proposed structures for ion codes are in Scheme II
[2] Relative intensity


m+
m/e 339 (343)
(a)
m/e 297 (301)
(c)
m/e 254 (258)
m+
m/e 339 (343)
(b)
m/e 255 (259)
(e)
m/e 136 (140)
(f)
m/e 133 (137)
(d)
m/e 108 (112)

SCHEME II

Structurally significant decomposition ions occur at m/e 136 (e) and 133 (f). They are shifted to m/e 140 and 137, respectively, for the d_4-product and therefore retain the aromatic protons. The initial step for the formation of m/e 136 is the abstraction of a proton from an acetyl group, followed by cleavage of the aromatic C-N bond. The formation of m/e 133 is much more complex and probably involves initial cleavage of the O - C(=O) - CH_3 and then loss of the pentafluoropropionyl moiety leaving the elements indicated in Scheme II. The formation of an isobaric benzyne type ion by a McLafferty abstraction of an aromatic proton was ruled out by the shift of m/e 133 to 137 in diacetylaminophenol-d_4 which indicated all four aromatic protons were retained in this ion structure.

ACKNOWLEDGMENT

This work was partially funded by grants from the National Institutes of Health NS 09199 and a Career Development Award HD 00128 (R.C.M.), and Pharmaceutical Manufacturers Career Development Award (C.R.F.).

REFERENCES

1. Mitchell J.R., Jollow D.J., Potter W.Z., Davis D.C., Gillette J.R. and Brody B.B. - J. Pharm. Exp. Ther. 187: 185 (1973).
2. Baty J.D. and Robinson P.R. - Clin. Pharm. Ther. 21: 177 (1977).
3. Bellamy L.J. - The Infra-red Spectra of Complex Molecules, 2nd edition, Methuen and Co., Ltd., London, 1958.
4. Merck Index, 8th edition, Merck and Co., p 5, 1968.