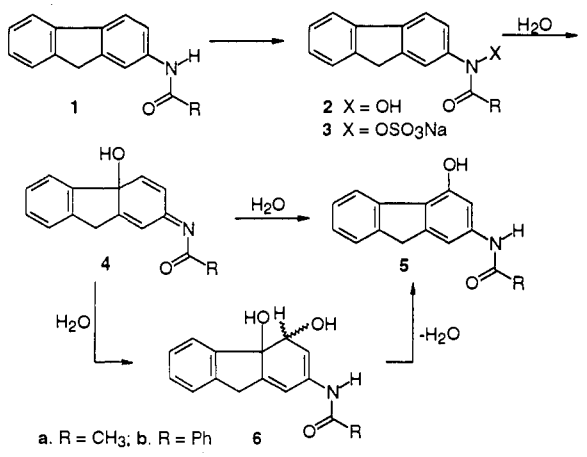
Model Studies on the Reactions of *N*-Acetyl-2-aminofluorene Metabolites. An Intramolecular Oxygen Migration from a *N*-Benzoyl Quinol Imine Intermediate†

Tim N. Biggs and John S. Swenton*

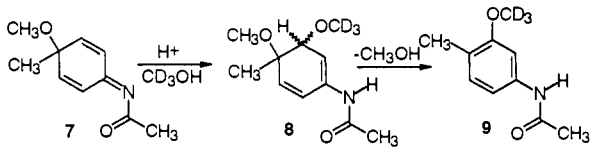
Department of Chemistry
The Ohio State University
140 West 18th Avenue
Columbus, Ohio 43210

Received August 10, 1993

Understanding the chemistry of metabolites of carcinogenic compounds is critical for establishing their mode of action. The metabolism of the carcinogen *N*-acetyl-2-aminofluorene (**1a**, R = CH_3) has been extensively studied.[1] Oxidation of **1a** in the liver by cytochrome P-450 produces the *N*-hydroxyl derivative, **2a**, which is then enzymatically sulfonated to give **3a**.[2] The quinol imine **4a** formed from **3a** is thought to be one of the major metabolites of **1a**.[3,4] Although **4a** has never been isolated, reactions have been designed to generate **4a** in situ.[3a,c,4] These studies have led to detection[3c] of an intermediate assigned as **4a** and to characterization of the products from these

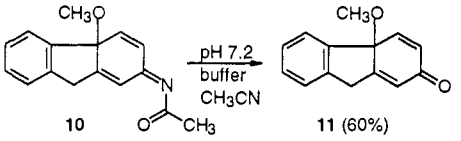


reactions.[3a,c,5] The mechanism for formation of the hydroxyl derivative **5a** has attracted much attention.[3] Originally the structure assignment for **5a** was incorrect.[6] Scribner[3a] reassigned the structure and suggested that this unusual conversion resulted from 1,4-addition of water to **4a** followed by aromatization, **4a** → **6a** → **5a**. In a subsequent mechanistic study, 1,4-methanol addition products **8** were isolated from an acid-catalyzed reaction of methanol and **7**.[3b] The methanol adducts **8** subsequently



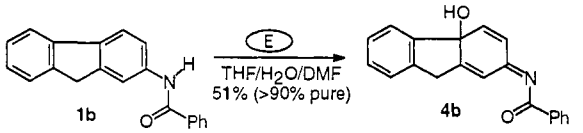
underwent aromatization by loss of methanol, **8** → **9**. This reaction sequence **7** → **8** → **9** was offered as support for the **4a** → **6a** → **5a** pathway proposed by Scribner.[3b] In addition, Michael addition products of **4a** have been implicated in kinetic studies with eventual formation of **5a**.[3c] We report here that the *N*-benzoyl analogue **4b** (R = Ph) is converted to a hydroxyl derivative (**5b**) in aqueous buffered solution (pH 7.2) at 40 °C. However, the retention of a labeled oxygen substituent and the failure to incorporate external oxygen from $H_2{}^{18}O$ in the **4b** → **5b** conversion is inconsistent with the major reaction pathway being **4b** → **6b** → **5b**.

We have been interested in preparing quinol imines such as **4a**[7] so that the proposed reaction pathways of these elusive intermediates can be studied under well-defined conditions. Although a variety of quinols and quinol esters can be prepared via anodic oxidation of the corresponding amides,[7] we could not detect the presence of **4a** under a variety of low-temperature anodic oxidation conditions.[7e] Undoubtedly, the high reactivity of the *N*-acetyl function[7d] was partly responsible for the lability of **4a**. However, **10**, the methoxyl analogue of **4a**, could be prepared via anodic oxidation.[7e] When **10** was allowed to react in an acetonitrile/pH 7.2 buffer solution at 40 °C for 2.5 h, **11** (60%) was the major product.[8] The formation of **11** contrasts with the formation of



5a, the product formed in reactions which generate **4a**. In these reactions, dienones analogous to **11** were not reported, and **5a** was obtained as a major product. It was surprising that the methoxyl analogue **10** would give a markedly different hydrolysis product than the hydroxyl derivative **4a**.[3a,c] *Could the conversion* **4** → **5** *be intramolecular and not proceed via the intermediacy of* **6** *as has been suggested*?

Although **4a** could not be prepared, after considerable experimentation we were able to prepare the benzoyl derivative **4b** by anodic oxidation of **1b**.[7e] The successful isolation of **4b** is



undoubtedly due to the increased stability of the imine linkage in this benzoyl derivative.[7d] Although **4b** could be obtained in acceptable yield, its lability prevented its isolation in analytically pure form. However, this quinol imine is an excellent system for investigating the course of the **4** → **5** conversion.

† Dedicated to the memory of Melvin S. Newman, a valued colleague and superb scientist.

(1) (a) Seal, U. S.; Gutmann, H. R. *J. Biol. Chem.* **1959**, *234*, 648. (b) Thorgeirsson, S. S.; Levitt, D. S.; Smith, C. L. In *Microsomes, Drug Oxidations, and Chemical Carcinogenesis*; Coon, M. J., Conney, A. H., Estabrook, R. W., Gelboin, H. V., Gillette, J. R., O'Brien, P. J., Eds.; Academic Press: New York, 1980; Vol. II, Chapter 7. (c) Lenk, W.; Rosenbauer-Thilmann, R. In *Microsomes, Drug Oxidations, and Chemical Carcinogenesis*; Coon, M. J., Conney, A. H., Estabrook, R. W., Gelboin, H. V., Gillette, J. R., O'Brien, P. J., Eds.; Academic Press: New York, 1980; Vol. II, Chapter 7. (d) Hara, E.; Kawajiri, K.; Gotoh, O.; Tagashira, Y. In *Microsomes, Drug Oxidations, and Drug Toxicity*; Sato, R., Kato, R., Eds.; Japan Scientific Societies Press: Tokyo, 1982; p 533. (e) Thorgeirsson, S. S. In *Microsomes and Drug Oxidations*; Boobis, A. R., Caldwell, J., DeMatheis, F., Elcombe, C. R., Eds.; Taylor and Francis: London, 1985; p 320.

(2) (a) Gorrod, J. W. In *Microsomes, Drug Oxidations, and Drug Toxicity*; Sato, R., Kato, R., Eds.; Japan Scientific Societies Press: Tokyo, 1982; p 621. (b) Kriek, E. *Biochim. Biophys. Acta* **1974**, *355*, 177.

(3) (a) Scribner, J. D. *J. Am. Chem. Soc.* **1977**, *99*, 7383. (b) Gassman, P. G.; Granrud, J. E. *J. Am. Chem. Soc.* **1984**, *106*, 2448. (c) Panda, M.; Novak, M.; Magonski, J. *J. Am. Chem. Soc.* **1989**, *111*, 4524.

(4) For discussions and leading references of nitrenium ion involvement in the **4** → **5** transformation, see: (a) Fishbein, J. C.; McClelland, R. A. *J. Am. Chem. Soc.* **1987**, *109*, 2824. (b) Campbell, J. J.; Glover, S. A.; Rowbottom, C. A. *Tetrahedron Lett.* **1990**, *31*, 5377.

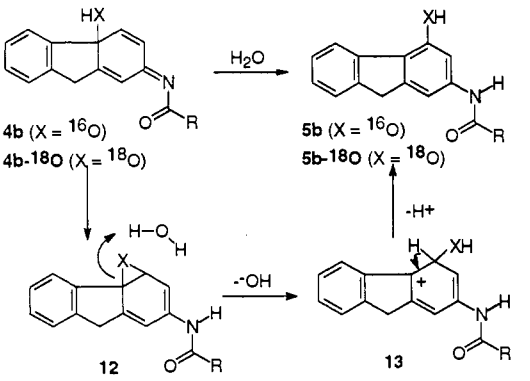
(5) (a) Beland, F. A.; Miller, D. W.; Mitchum, R. K. *J. Chem. Soc., Chem. Commun.* **1983**, 30. (b) Smith, B. A.; Springfield, J. R.; Gutman, H. R. *Carcinogenesis* **1986**, *7*, 405. (c) Meerman, J. H. N.; Beland, F. A.; Ketterer, B.; Srai, S. K. S.; Bruins, A. P.; Mulder, G. J. *Chem.-Biol. Interact.* **1982**, *39*, 149. (d) Gosold, F. Boche, G. *Angew. Chem., Int. Ed. Engl.* **1990**, *29*, 63.

(6) Gutmann, H. R.; Malejka-Giganti, D.; McIver, R. *J. Chromatogr.* **1975**, *115*, 71–78.

0002-7863/93/1515-10416$04.00/0

When **4b** was reacted in 15:1 acetonitrile/$H_2{}^{18}O$ (>95% ^{18}O) at 40 °C,[9] workup and chromatography on silica gel gave **5b** (66%), which by mass spectral analysis contained no ^{18}O.[10] The product obtained from a second reaction under identical conditions gave the same mass spectral result. Although this result unquestionably rules out the **4b** → **6b** → **5b** pathway, a confirmation of this result was desired. Anodic oxidation of **1b** in tetrahydrofuran/$H_2{}^{18}O$ (>95% ^{18}O) gave **4b**-^{18}O (50%, 74% ^{18}O).[11] Reaction of **4b**-^{18}O in 1:1 acetonitrile/$H_2{}^{16}O$ followed by workup and silica gel chromatography gave **5b**-^{18}O (70%, 78% ^{18}O). A duplicate of the anodic oxidation and hydrolysis steps gave the same results. *Thus, it is unequivocally demonstrated that the* **4b** → **6b** → **5b** *pathway is not operative for the N-benzoyl derivative. It is very unlikely that a different reaction mechanism would be followed for the* **4a** → **6a** → **5a** *reaction.*

This study strongly suggests that the mechanism first suggested by Scribner[3a] and later supported by the studies of Gassman[3b] for the formation of **5a** from *N*-acetyl-2-aminofluorene is not operating. However, the detection of an intermediate assigned as **4a** by Novak coupled with the rearrangement of **4b** → **5b** reported here supports the intermediacy of the nonisolable **4a** in the formation of **5a**. A possible reaction sequence for the observed labeling results is outlined below **4** → **12** → **13** → **5**. The oxa norcaradine **12** is analogous to intermediates postulated in the



NIH-shift reaction;[12] however, further mechanistic discussion is deferred until kinetic studies are complete. We are unaware of formal hydroxyl migrations having been reported previously in the chemistry of either quinols or quinol imines. Most often, aryl group migration is the favored reaction pathway in 4-aryl-substituted quinol imines and their ethers.[7b,d] However, this particular pathway is probably less favorable in fluorene systems such as **4**, since the stereoelectronic requirements for aryl migration may not be favorable and intermediates arising from aryl migration would afford strained ring systems. The generality of this type of hydroxyl migration in other systems remains to be established.

Acknowledgment. We thank the National Science Foundation for purchase of the electrochemical equipment and the National Institutes of Health, Grant GM 36592-04, for financial support of this work.

Supplementary Material Available: Experimental procedures, ^1H NMR spectra of all new compounds, and mass spectra employed in the ^{18}O analyses (15 pages). This material is contained in many libraries on microfiche, immediately follows the article in the microfilm version of the journal, and can be ordered from the ACS. Ordering information is given on any current masthead page.

(7) (a) Chen, C.-P.; Chou, C.-T.; Swenton, J. S. *J. Am. Chem. Soc.* **1987**, *109*, 946. (b) Swenton, J. S.; Bonke, B. R.; Chen, C.-P.; Chou, C.-T. *J. Org. Chem.* **1989**, *54*, 51. (c) Clark, W. M.; Swenton, J. S. *J. Org. Chem.* **1990**, *55*, 3969. (d) Novak, M.; Helmick, J. S.; Oberlies, N.; Rangappa, K. S.; Clark, W. M.; Swenton, J. S. *J. Org. Chem.* **1993**, *58*, 867–878. (e) Swenton, J. S.; Biggs, T. N.; Clark, W. N. *J. Org. Chem.* **1993**, *58*, 5607.

(8) Although a number of minor products were formed, as ascertained by the TLC of the reaction mixture, nothing occurred at the R_f of **5a**. We thank Professor Michael Novak for an authentic sample of **5a**.

(9) The $H_2{}^{18}O$ was obtained from Isotec Inc. and was assayed as 95.8 atom % ^{18}O.

(10) The molecular ion peak of **5b**,**5b**-^{18}O was used for analysis of the reaction mixtures and the actual spectra are presented in the supplementary material. The amount of ^{18}O in the samples was determined from the ratio of the respective molecular ion peaks. No appreciable changes in composition resulted from corrections due to the (M + 2) peaks. The ^{18}O compositions for **5b**,**5b**-^{18}O are thought to be accurate to at least ±4%.

(11) The ^{18}O content of **4b**-^{18}O was assayed from the ratios of the molecular ion peaks in the mass spectrum. This peak was less intense than those for **5b**,**5b**-^{18}O, and we estimate the accuracy of the ^{18}O compositions as at least ±8%. The lower isotopic content of **4b**-^{18}O relative to starting $H_2{}^{18}O$ could have arisen from contamination with $H_2{}^{16}O$ during the small-scale, low-temperature anodic oxidation.

(12) Guroff, G.; Daly, J. W.; Jerina, D. M.; Renson, J.; Witkop, B.; Undenfriend, S. *Science* **1967**, *157*, 1524.