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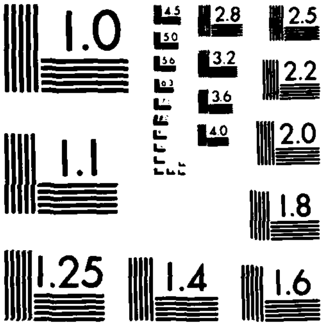

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THE UNIVERSITY OF OKLAHOMA

GRADUATE COLLEGE

SYNTHESIS OF POTENTIAL METABOLITES IN THE 1,2,3,4 AND 5,6,7,8 BENZO RING POSITIONS OF THE POLYCYCLIC AROMATIC HYDROCARBON BENZO(G)CHRYSENE

A DISSERTATION

SUBMITTED TO THE GRADUATE FACULTY

in partial fulfillment of the requirements for the

degree of

DOCTOR OF PHILOSOPHY

By

CLIFFORD MARTIN UTERMOEHLEN

Norman, Oklahoma

1986

DEDICATION

Dedicated to Beverly, Kathryn, and C. Gregory, whose
love and patience made this work possible

SYNTHESIS OF POTENTIAL METABOLITES IN THE 1,2,3,4 AND 5,6,7,8 BENZO RING POSITIONS OF THE POLYCYCLIC AROMATIC HYDROCARBON BENZO(G)CHRYSENE

A DISSERTATION

APPROVED FOR THE DEPARTMENT OF CHEMISTRY

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TABLE OF CONTENTS

TABLE OF CONTENTS (continued)

LIST OF TABLES

LIST OF TABLES (continued)

LIST OF FIGURES

LIST OF FIGURES (continued)

LIST OF FIGURES (continued)

LIST OF FIGURES (continued)

ABSTRACT

SYNTHESIS OF POTENTIAL METABOLITES IN THE 1,2,3,4 AND 5,6,7,8 BENZO RING POSITIONS OF THE POLYCYCLIC AROMATIC HYDROCARBON BENZO(G)CHRYSENE

BY CLIFFORD M. UTERMOEHLEN

Polycyclic aromatic hydrocarbons (PAH) are unavoidable pollutants of our environment. Studies have linked cancers directly to contact with these contaminants. Within the past ten years evidence supports the finding that the "ultimate carcinogen" in the metabolic chain of a PAH is the bay region diol epoxide. The most studied PAH is benzo(a)pyrene.

Recent tests indicate benzo(g)chrysene (XL), another PAH, is also a potent carcinogen. Research efforts in this dissertation involve the synthesis of potential metabolites of this PAH. Because XL has two fjord and three bay region benzylic positions, it will permit assessment of steric factors associated with biological activity. Recent publications include vicinal hydroxyl orientation effects in their structure reactivity discussions.

This dissertation describes the total syntheses of (±)-3,4-dihydroxy-1,2-epoxy-1,2,3,4-tetrahydrobenzo(g)chrysene isomers 1 and 2. It also includes synthesis of the 1,2-dihydro and 3,4-dihydro-3,4-dihydroxy derivatives. Syntheses associated with the 5,6,7,8 benzo ring include

7,8-dihydro; 5,6-dihydro-5,6-dihydroxy; and 5,6,7,8-tetrahydro-5,6-epoxide. A synthesis of the parent PAH, benzo(g)chrysene is also included.

SYNTHESIS OF POTENTIAL METABOLITES IN THE 1,2,3,4 AND 5,6,7,8 BENZO RING POSITIONS OF THE POLYCYCLIC AROMATIC HYDROCARBON BENZO(G)CHRYSENE

CHAPTER 1

BACKGROUND AND HISTORY

Polycyclic aromatic hydrocarbons (PAH) are unavoidable environmental pollutants in our society. Many of these are carcinogenic. Some studies indicate environmental carcinogens contribute to more than 50% of human cancers (1). Benzo(a)pyrene, a PAH, is produced in the United States on the order of 1,000 tons per year. Sources include power generation, refuse burning, coke production and automobile emissions. Once in the air, PAH can enter into the food chain and spread to animals and humans (2).

There are several current reviews available that detail the history of PAH's, their link with cancer, and the metabolic process (1,2,3). This paper highlights some of these topics. These reviews also point out that a correlation exists between PAH structure and activity. Several theories have been offered. However, studies are still required to test these theories and better explain the anomalies in these correlations and to determine if there are other yet unknown factors that play important roles in PAH carcinogenesis.

Environmental hazards were correlated with cancer as early as 1775. Percival Pott attributed the high incidence of scrotum cancer in English chimney sweepers with their exposure to soot and their poor hygiene habits. The industrial revolution in Europe further showed job related cancers in coal tar, shale oil industries and automatic machine shops (use of lubricating oils) (1).

In 1915, two Japanese workers, Yamagiwa and Ichikawa, provided direct evidence that coal tar caused cancers. They painted rabbit ears with coal tar and induced tumors. This was later applied to mouse skin with similar results (1,2,3).

These early results expanded activity toward the isolation of components responsible for carcinogenicity. A major breakthrough was the observation that carcinogenic fractions of coal tar gave a distinct fluorescence spectrum. Kennaway, Mayneord, and Hieger used that characteristic in following carcinogenic activity (3). In 1930, a gas works plant distilled two tons of pitch for laboratory research. After three years of isolation and purification, Cook, Hewett, and Hieger were able to identify benzo(a)pyrene as the carcinogenic compound of coal tar (4).

With that discovery as the backbone in PAH research, the field rapidly expanded into the study of a number of synthetic hydrocarbons - some of which were carcinogenic and others inactive. Questions began arising as to what aspects of these molecules caused them to be carcinogenic while others were not. In 1971 the National Cancer Act established a U.S. strategy toward cancer to "develop a means to prevent cancer, cure it when it can't be prevented and achieve long term survival when it can't be cured" (1).

Current theory describes the nature of carcinogenesis in two general steps. These are called initiation and promotion.

Initiators are chemically reactive toward nucleophilic sites in the cells. They can be reactive in their basic form or metabolically activated. Initiators form an essentially irreversible bond with a cellular site and are considered mutagenic.

Promoters are reactive without metabolism. When a promoter is applied to skin without initiation, no measurable increase in tumor activity occurs. A promoter is applied in small amounts over a long period (see Figure 1.1). An interesting aspect of promotion is its somewhat reversible action. At least, if applied at wide intervals or in insufficient amounts, it was less effective than doses closely spaced (5).

Figure 1.1

Diagram of Initiation and Promotion Sequence (5)

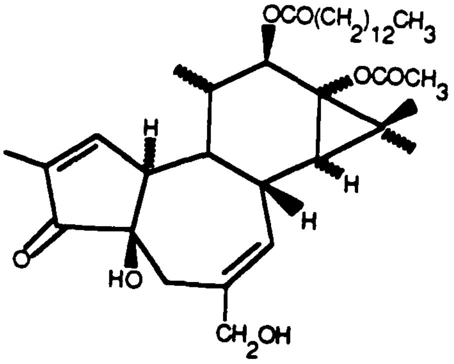
Time line	Tumors
[I]	0
[I][----P-----]	High
[I][--1 yr interval--][--P--]	High
[P----][I]	0
[P----------]	0

I=initiator (50ug 7,12-dimethyl benz(a)anthracene)

P=promoter (10ug croton oil, 2x/week)

This two stage process doesn't prohibit existence of a complete carcinogen or co-carcinogen that serves as both promoter and initiator. Both components may also be present in one source and introduced simultaneously. A very effective promoter used to produce tumors on mice skin is oil of croton seeds. The active component is 12-O-tetradecanoylphorbol-13-acetate whose structure is given below (5).

Figure 1.2



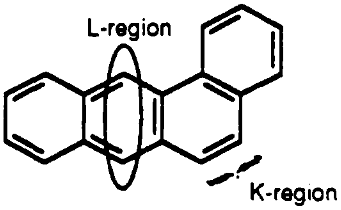
12-O-tetradecanoylphorbol-13-acetate

With the large number of polycyclic aromatic hydrocarbons (PAH) being studied for carcinogenic activity, researchers began correlating structural features of molecules with activity (3,6,7). For example, linear PAH, such as anthracene or naphthacene are inactive as tumor initiators. However, molecules with an area of high pi electron density, such as benzo(a)pyrene, may be active. Schmidt was among the first to note this relationship. He believed this high pi electron density related to biological activity. Pullman, in 1945, noted that active compounds contained angular benzo rings. She introduced the terminology "K-region" to refer to the region of the molecule with the highest double bond character (3). Figure 1.3 illustrates these regions.

It was clear that there were more factors relating the structure of PAH with carcinogenic activity than the existence of a K-region. Pullman suggested that if there also existed an active L-region, this could deactivate carcinogenic activity (3). Benzo(a)pyrene fits the description as being active while benz(a)anthracene,with an L-region, is only mildly active. Pullman believed the site of carcinogenic activity was the K-region. However later studies revealed the K-region arene oxides to be less carcinogenic than the parent hydrocarbon (8).

Figure 1.3

K and L Regions



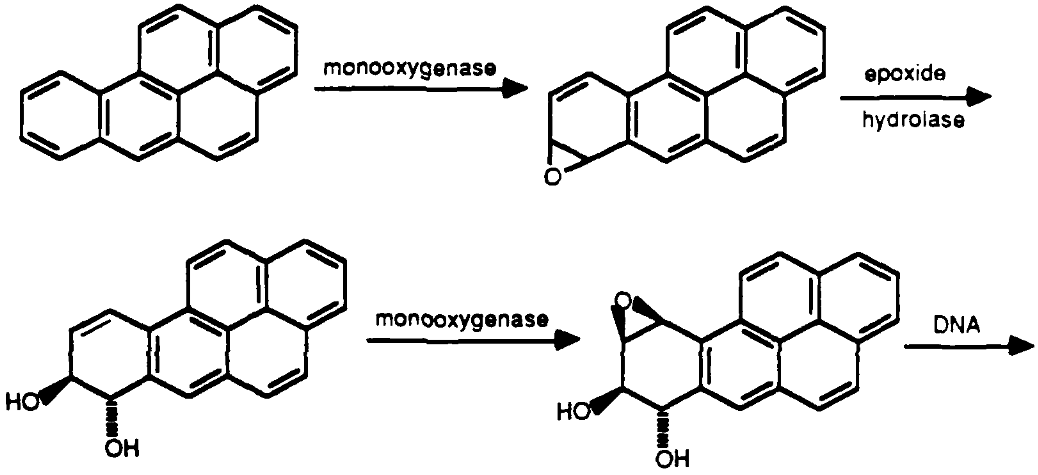
Major metabolites have been identified as trans-dihydrodiols and phenols with other components (9). Boyland suggested that epoxides were sufficiently active and could be the reactive intermediate species responsible for the activity of PAH (10).

Workers then began trying to identify the actual metabolite responsible for binding to DNA. Borgen, in 1973, reported that upon metabolism with microsomal preparations, the 7,8-dihydrodiol of benzo(a)pyrene was more extensively bound to DNA than the parent PAH. Sims, in 1974, reported that the dihydro diol epoxide was responsible for DNA binding (3).

The principal path for metabolic activation of benzo(a)pyrene is shown in Figure 1.4. By 1975, this mechanism of activation was strongly implicated. Work by Jerina, et. al. led to the conclusion that the diol epoxide was an ultimate carcinogen (11).

Figure 1.4 (9)

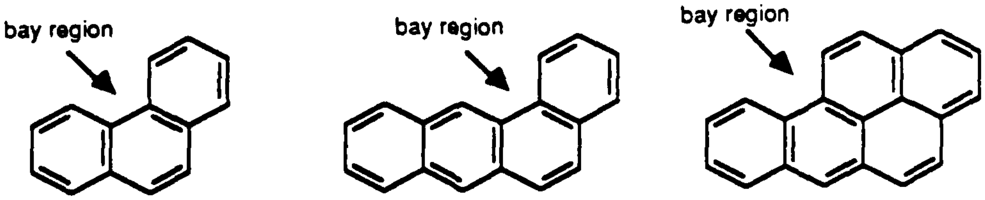
Benzo(a)Pyrene Metabolic Activation



Jerina and Daly presented the "bay region" theory in 1976 as a method of selecting the active site of a PAH for biological activity. The bay region is the sterically hindered portion of a polycyclic aromatic hydrocarbon that

Figure 1.5

PAH Bay Region



is created by a fused angular ring structure (12). Phenanthrene is the smallest PAH with a bay region.

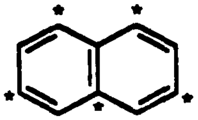
The "Bay Region" theory came from work on benz(a)anthracene derivatives and calculated reactivities at the benzylic positions of tetrahydro derivatives within the molecule (13). With the identification of the ultimate metabolic form as the *trans*-vicinal dihydroxy epoxide, one rapidly discerns that PAHs have numerous sites available for metabolism. This theory predicts the "bay region diol epoxides would be the important ultimate carcinogens *when they were metabolically formed* from a tumorigenic PAH" (13).

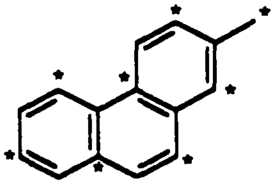
A precise mechanism for the PAH metabolite binding with DNA is not yet defined. However, the model generally used opens the epoxide, forming a benzylic carbocation. This then binds with an amine site of DNA (8).

Lowe and Silverman pointed out that the bay region of a PAH is the only location that can produce a triol carbocation adjacent to a ring fusion. These are more stable than other carbocations because the higher energy molecular orbitals show small coefficients at fusion sites and large coefficients at atoms adjacent to the fusion (14).

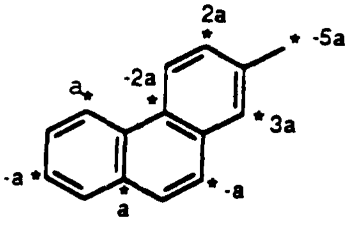
The bay region theory examines the change in the pi energy that occurs upon forming a benzylic carbocation. By assuming the reactivity is related primarily to the pi system and changes to that pi system, the perturbational molecular orbital method permits easy calculations that predict which diol epoxide metabolite of a polycyclic aromatic hydrocarbon will be most reactive and its relative activity (12).

Dewar's perturbational molecular orbital method is used to calculate molecular orbital coefficients for conjugated systems. A conjugated system can be termed alternant if it can be divided into a set of starred and unstarred atoms so that no two are adjacent (15).





These are further divided into odd or even alternant systems depending on the number of conjugated atoms. An odd alternant system can be set up so that the sum of the coefficients around each unstarred position is 0. In addition, unstarred atoms have 0 as coefficients. An example is shown below:



Normalization of the coefficients provides the following numerical values:

$$1=(-5a)^2+(3a)^2+(-a)^2+a^2+(-a)^2+a^2+(-2a)^2+(2a)^2$$

$$=46a^2$$

$$a=(1/46)^{.5}=.147$$

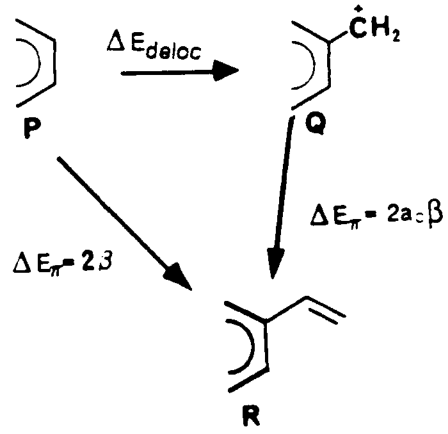
"Union" is another concept in the PMO method. Union is used to describe a combination of fragments to make an alternant hydrocarbon. The change in energy for this union is given by:

$$\delta E=\Sigma 2a_{or}b_{os}\beta_{rs}$$

where a_{or} and b_{os} are coefficients of the nonbonding molecular orbitals of r and s. Since methyl can be considered the limiting case, its coefficient is "1". Therefore the union with methyl provides a change in energy (15):

$$\delta E=2a_{or}\beta_{rs}$$

The bay region theory involves delocalization energy, or ΔE_{deloc}. This is defined by Dewar as the difference in delocalization energy between the parent hydrocarbon and the corresponding carbocation (15). Consider the cycle:



The parent PAH, P, can be made a carbocation, Q, by the union of P with methyl. Molecule Q can now be converted to another molecule, R, by union with another methyl. The pi energy change for Q going to R is then given by:

$$\delta E = 2a_0\beta$$

(a_0 is the coefficient of the binding atom of Q)

Molecule R can also be formed by the union of P with ethylene. This union forms a localized pi bond, or the difference in energy, by Huckel, is 2β (15).

$$\Delta E_{PR}=2\beta$$

In order to conserve energy, $\Delta E_{PR}=\Delta E_{PQ}+\Delta E_{QR}$

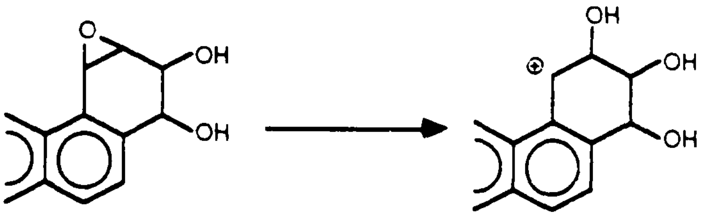
$$\text{or, } \Delta E_{deloc}=\Delta E_{PR}-\Delta E_{QR}$$

$$=2\beta-2a_0\beta$$

$$\Delta E_{deloc}=2\beta(1-a_0)$$

This expression for energy is used throughout bay region theory discussions.

The actual reaction intended for the bay region theory is shown below:

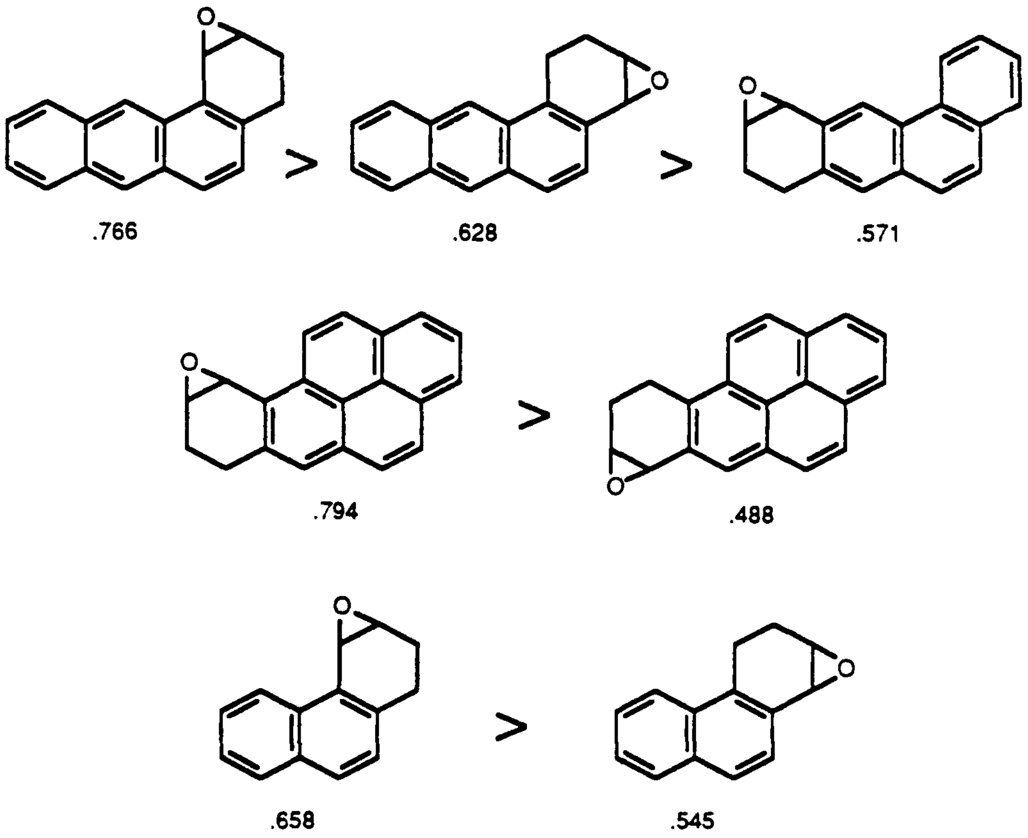


However, it neglects any effect hydroxyls may have on the epoxide opening. Tetrahydro epoxides (cf. Figure 1.6) give excellent correlation when measured reactivity (hydrolysis kinetics) is compared to $\Delta E_{deloc}/\beta$ (12).

In addition, where a variety of tetrahydro epoxides are tested within the same parent ring system, $\Delta E_{deloc}/\beta$ provides correct ordering for mutagenicity against S. typhimurium strain TA100. This is shown in Figure 1.6 (12).

Figure 1.6

$\Delta E_{deloc}/\beta$ and Relative Mutagenicity Results of Tetrahydro Epoxides with S. Typhimurium (12)



Although theoretical approaches to PAH and carcinogenesis have been criticized (16), it encourages research into the area of predicting carcinogenicity from chemical structure alone. The bay region theory is being tested in this respect. Twenty hydrocarbons are listed in Table 1.1 by decreasing delocalization energy. The listing provides a reasonable ranking. However, there are a large number of variables in biological systems, including extent of metabolism to the bay region diol epoxide, that must affect correlation. Also, the PAH metabolite may have additional characteristics not yet identified that play a role in carcinogenicity.

Recent reviews and publications (12,17) expanded the bay region theory by classifying diol epoxides in terms of series and groups within a series. New correlation charts are now available and provide good results.

There are two diastereomeric sets of *trans*-diol epoxides. These are defined by the relationship of the epoxide oxygen to the benzylic hydroxyl. If the benzylic hydroxyl is on the same side of the aromatic nucleus plane, the diol epoxide is called *cis* or series 1. When the benzylic hydroxyl is on the opposite side of the molecular plane, the diol epoxide is *trans* or series 2. Early studies indicated that isomer 1 is the more reactive diol epoxide in hydrolysis reactions, yet isomer 2 is more biologically active (18,19).

Series 1 and 2 epoxides are further divided by hindered and non-hindered benzylic hydroxyls. For series 1, if the benzylic hydroxyl is in a bay region, the diaxial

Table 1.1*

ΔE_{deloc} and Carcinogenicity of PAH

PAH	$\Delta E_{deloc}/\beta$	Bay Region	Carcino-genicity**
dibenzo(a,i)pyrene	0.866	yes	++++
dibenzo(a,h)pyrene	0.845	yes	++++
tribenzo(a,e,i)pyrene	0.818	yes	++
dibenzo(a,l)pyrene	0.800	yes	++++
benzo(a)pyrene	0.794	yes	++++
dibenzo(a,e)pyrene	0.775	yes	+++
benz(a)anthracene	0.766	yes	+
dibenz(a,h)anthracene	0.738	yes	++
dibenz(a,j)anthracene	0.722	yes	+
dibenz(a,c)anthracene	0.722	yes	+
benzo(g)chrysene	0.719	yes	++
benzo(e)pyrene	0.714	yes	+
triphenylene	0.664	yes	-
picene	0.662	yes	-
phenanthrene	0.658	yes	-
benzo(b)chrysene	0.647	no	-
chrysene	0.639	yes	+
tetracene	0.628	no	-
benzo(c)phenanthrene	0.600	yes	+
anthracene	0.545	no	-

* Extracted from reference 14.

** -, inactive; +, slightly active; ++, fairly active; +++, very active; ++++, extremely active.

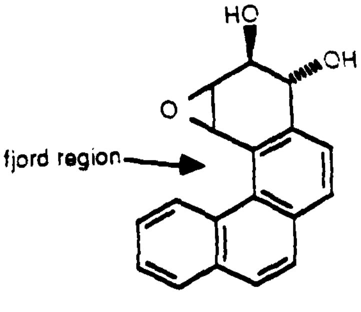
arrangement is strongly preferred even when the epoxide is hindered. If the hydroxyl is not in a bay region, the diaxial arrangement is slightly preferred (19,20).

In series 2 diol epoxides, there is a greater steric effect. In these cases, a bay region benzylic hydroxyl results in a diaxial conformation whereas a non-bay region hydroxyl results in a favored diequatorial arrangement (19,20). Taking into account this group division within each series, there is a correlation, both in chemical activity and biological activity, between increasing $\Delta E_{deloc}/\beta$ with rate and relativemutagenicity. Apparently, quasi diequatorial and quasi diaxial arrangements of the vicinal hydroxyls play important roles in how the PAH metabolite binds with DNA. Bay region diol epoxides thus far examined demonstrate tumorigenicity only when they prefer the quasi-diequatorial conformation (12).

Benzo(c)phenanthrene is unique among the PAH studied thus far. In this case, diol epoxides of series 1 and 2 prefer the quasi diequatorial conformation due to the steric crowding of the epoxide in what is termed the "fjord region" (Figure 1.7) (20).

Figure 1.7

Fjord Region

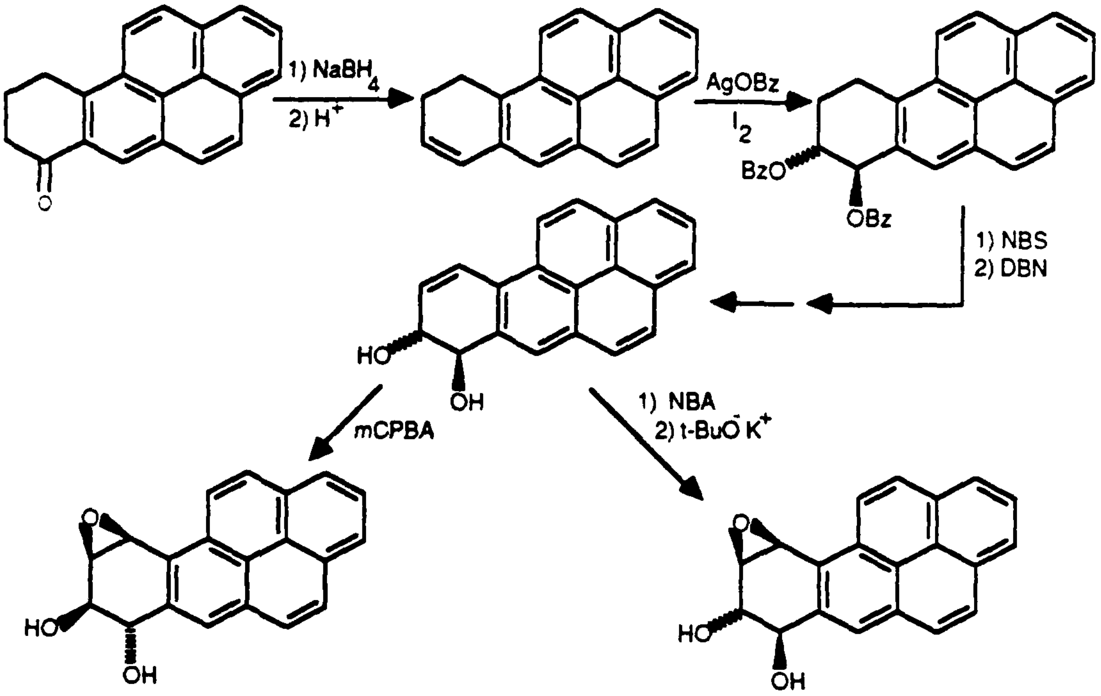


Mutagenicity and tumorigenicity is expected and, in fact, is close to that of benzo(a)pyrene in most cases (17,21). Because both epoxide series have quasi diequatorial hydroxyls, one would anticipate high tumor activity for each. However, recent tumor studies indicate that series 1 epoxide gave these results only on mouse skin painting. Epoxide -1 did not give the expected high tumorigenicity results in newborn mice like that of epoxide-2. The authors indicate that perhaps diequatorial hydroxyls don't activate the reaction with DNA, but in reality diaxial hydroxyls prohibit the reaction (17). It appears additional factors need to be considered to improve correlations with biological activity.

Thus far a number of dihydrodiols *have* been prepared and biological tests accomplished that confirm the basic premise of the bay region theory. These syntheses generally involve the sequence ketone to alkene to trans-diester. Following bromination, dehydrobromination and deprotection, the resulting dihydrodiol is oxidized to the diol epoxide. One example is shown for benzo(a)pyrene in Figure 1.8. Variations are also available that go directly from the tetrahydro dibenzoate to the dihydro dibenzoate or substitute the acetate ester for the benzoate. The particular PAH dictates the correct route for the best results.

Figure 1.8 (22)

Synthesis of Benzo(a)pyrene Diol Epoxide



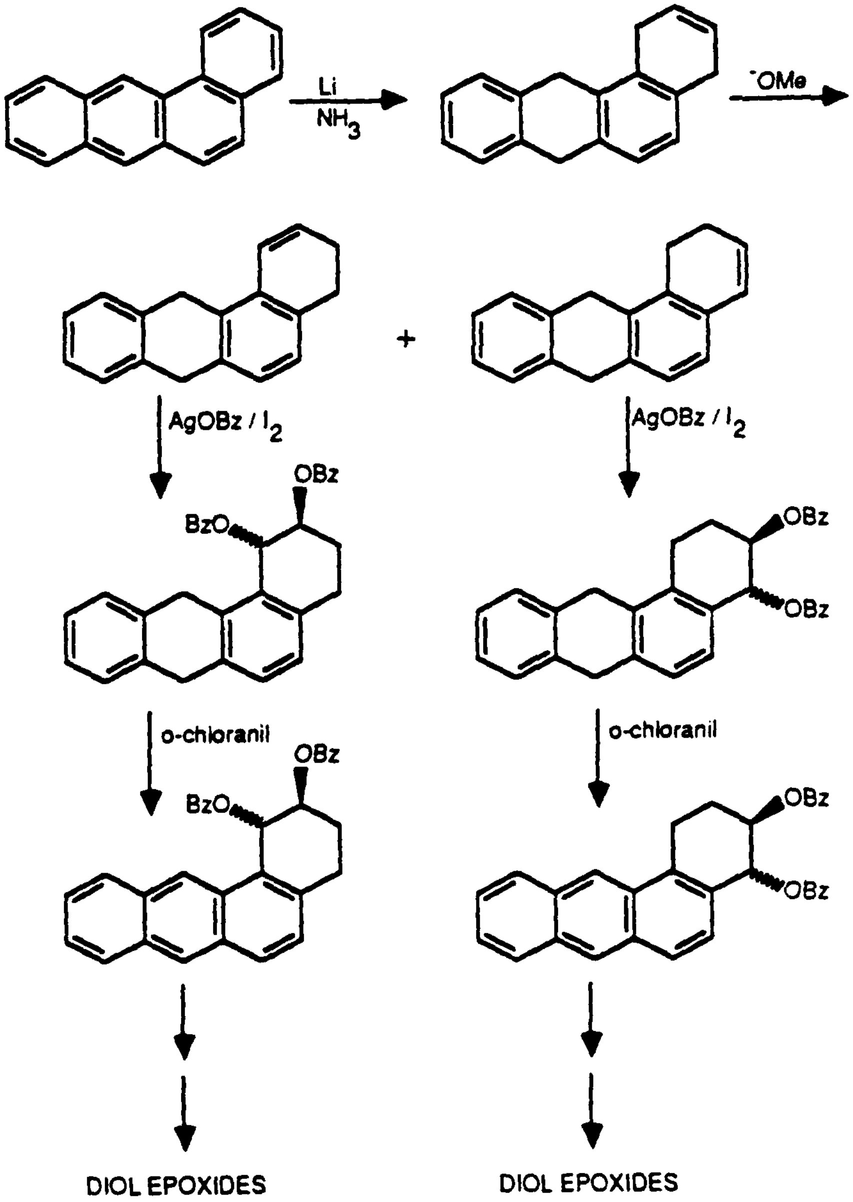
Another variation to diol epoxide synthesis starts with the parent hydrocarbon. In this method the PAH is reduced using dissolving metal and isomerized to conjugated forms. Following the Prévost reaction, the tetrahydro dibenzoate is ready for re-aromatization of the non-benzo ring (see Figure 1.9). Conversion to diol epoxides is similar to that described above. Other methods of reduction can be used to provide different results. Catalytic hydrogenation with platinum wire, for example, reduced the terminal ring rather than the angular benzo ring.

These methods generally describe the procedures reported thus far in the synthesis of PAH diol epoxides.

Details of these procedures and other variations are available (22).

Figure 1.9 (22)

Synthesis of Benz(a)anthracene Diol Epoxides from PAH



Current information on hindered diol epoxides is based on that available from benzo(c)phenanthrene and 5-methyl chrysene. Both of these molecules form highly hindered diol epoxides (Figure 1.10) and demonstrate higher tumorigenicity and mutagenicity (17) than anticipated by simple PMO calculations. Steric hindrance caused by the fjord region must be an important factor since the calculated value of $\Delta E_{deloc}/\beta$ for benzo(c)phenanthrene is 0.600 while benzo(a)pyrene is considerably higher, at 0.794.

Figure 1.10

Hindered Diol Epoxides

benzo(c)phenanthrene
diol epoxide

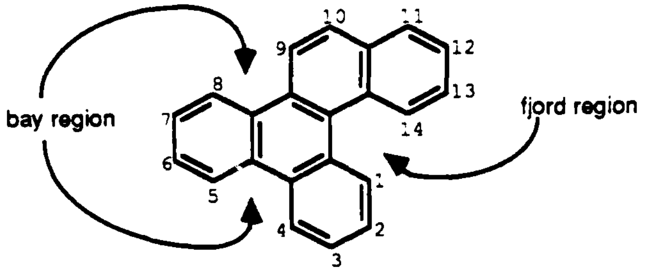
5-methyl chrysene
diol epoxide

Further examination into apparent exceptions to bay region predictions should help identify how these molecules do correlate. What is now known is that diaxial hydroxyl groups lead to reduced activity, and increased steric hindrance at the epoxide tends to increase activity. The latter result is based on benzo(c)phenanthrene studies.

This study initiates work toward better understanding how steric effects and hydroxyl orientation affects activity. In particular, our work centered on derivatives of the PAH benzo(g)chrysene, Figure 1.11.

Figure 1.11

Benzo(g)chrysene



Benzo(g)chrysene has two bay regions and one fjord region. Calculated values for $\Delta E_{deloc}/\beta$ for the tetrahydro benzo rings are: C_1(.623), C_4(.719), C_5(.667), C_8(.638), C_{11}(.545), and C_{14}(.586). Based on delocalization energy alone, one would predict the 1,2-diol-3,4-epoxide to be the most reactive toward biological systems. However, with the additional factor of hydroxyl orientation (based on steric reasons) the only diequatorial arrangement probably exists with the 11,12-diol-13,14-epoxide. Hence, that would then be predicted as the major mutagen and tumorigen of the various possible diol epoxides.

This work provides the synthesis of a number of possible metabolites of the PAH benzo(g)chrysene. In particular, benzo(g)chrysene-3,4-diol-1,2-epoxides, *trans*-3,4-dihydro-3,4-dihydroxybenzo(g)chrysene, *trans*-5,6-dihydroxy-5,6-dihydrobenzo(g)chrysene, and 5,6,7,8-tetrahydrobenzo(g)chrysene-5,6-epoxide. Chapter 2 describes the synthesis of the first two while chapter 3 describes the 5,6,7,8 derivatives. Based on similarities with other PAHs, structural implications of the potential metabolites are discussed. Biological testing will be performed in another laboratory (23).

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23. Metabolism studies of benzo(g)chrysene are being conducted at the National Institutes of Health, Bethesda, Md., under direction of Donald M. Jerina. Mutagenicity and tumorigenicity studies will be performed in the laboratory of Wayne Levin and Alexander W. Wood, Roche Institute of Molecular Biology, Nutley, New Jersey.

SYNTHESIS OF 3,4-DIHYDRODIOL-1,2-EPOXY-BENZO(G)-CHRYSENES AND RELATED 1,2,3,4 BENZO RING DERIVATIVES OF BENZO(G)CHRYSENE

CHAPTER 2

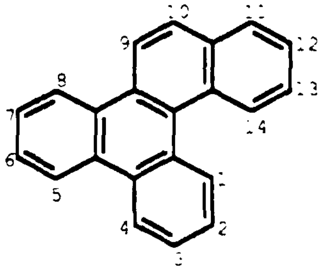
INTRODUCTION

This work consisted of the synthesis of dihydrodiol epoxides, dihydro diols, and dihydro derivatives in the 1,2,3,4 benzo ring of the carcinogen, benzo(g)chrysene. As was mentioned earlier, angular rings increase carcinogenic activity, most probably due to formation of a "bay region" diol epoxide.

Benzo(g)chrysene is an interesting molecule to test for biological activity. There are two bay regions as well

Figure 2.1

Benzo(g)chrysene



as a fjord region. This provides two fjord and three benzylic bay region positions for epoxide derivatives.

Table 2.1

Calculated Resonance Energy Stabilization

Benzylic position	Type position	Resonance energy* (ΔE/β)
1	fjord	.623
4	bay	.719
5	bay	.667
8	bay	.638
11	benzylic	.545
14	fjord	.586

*calculated by the PMO method (1).

The results of the calculations of resonance energy stabilization are shown in Table 2.1. Based solely on these calculations, one would predict epoxides at the 3,4; 5,6; and 7,8 positions to exhibit the greatest reactivity because of the stability of the carbocation generated at the site of epoxide opening. However, the conformation of the saturated ring apparently plays an important role in determining the carcinogenicity and tumorigenicity of these molecules.

Preliminary carcinogenicity tests have shown that benzo(g)chrysene, the parent hydrocarbon for these studies, is a potent carcinogen. One would anticipate that there is a metabolite of this hydrocarbon that produces cancers in preference to others. The structure of this ultimate metabolite for the molecule is under investigation in these laboratories. With the number of possibilities that exist in the molecule and the range of reactivity that is possible, benzo(g)chrysene is an excellent candidate to test structure-activity theories.

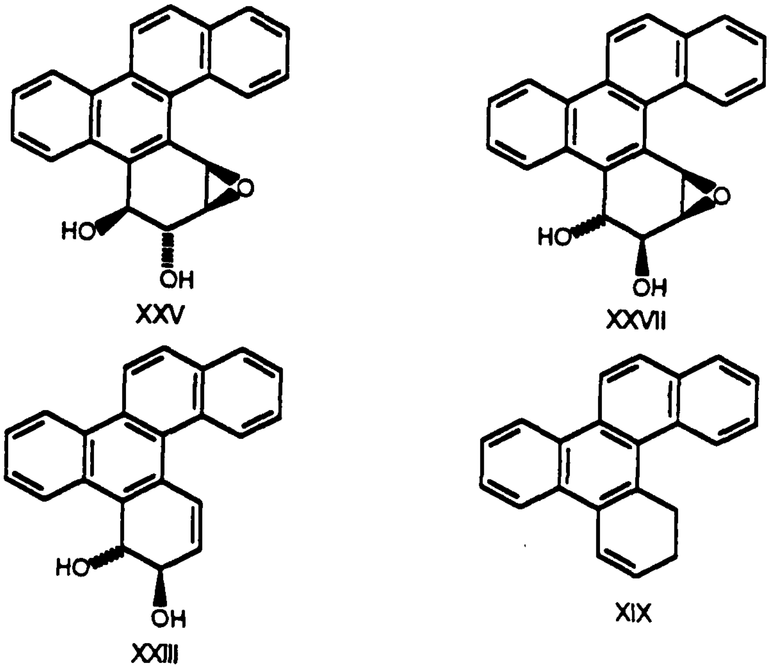
Although they do not have the highest calculated $\Delta E_{deloc/\beta}$, our interest centered on the 1,2 epoxide derivatives. As mentioned previously, benzo(c)phenanthrene exhibited high tumorigenicity when the epoxide was in the fjord region (2). In that case, however, the hydroxyls were quasi-diequatorial. Our project was to prepare a fjord diol epoxide and restrict the hydroxyls in a diaxial conformation. Biological testing will determine if the anticipated attenuation of activity occurs with the fjord as with the bay system.

RESULTS AND DISCUSSION

To provide material for testing, this research project involved the synthesis of several possible metabolites of benzo(g)chrysene. These molecules are shown in Figure 2.2. All compounds discussed in this paper are racemic unless otherwise indicated.

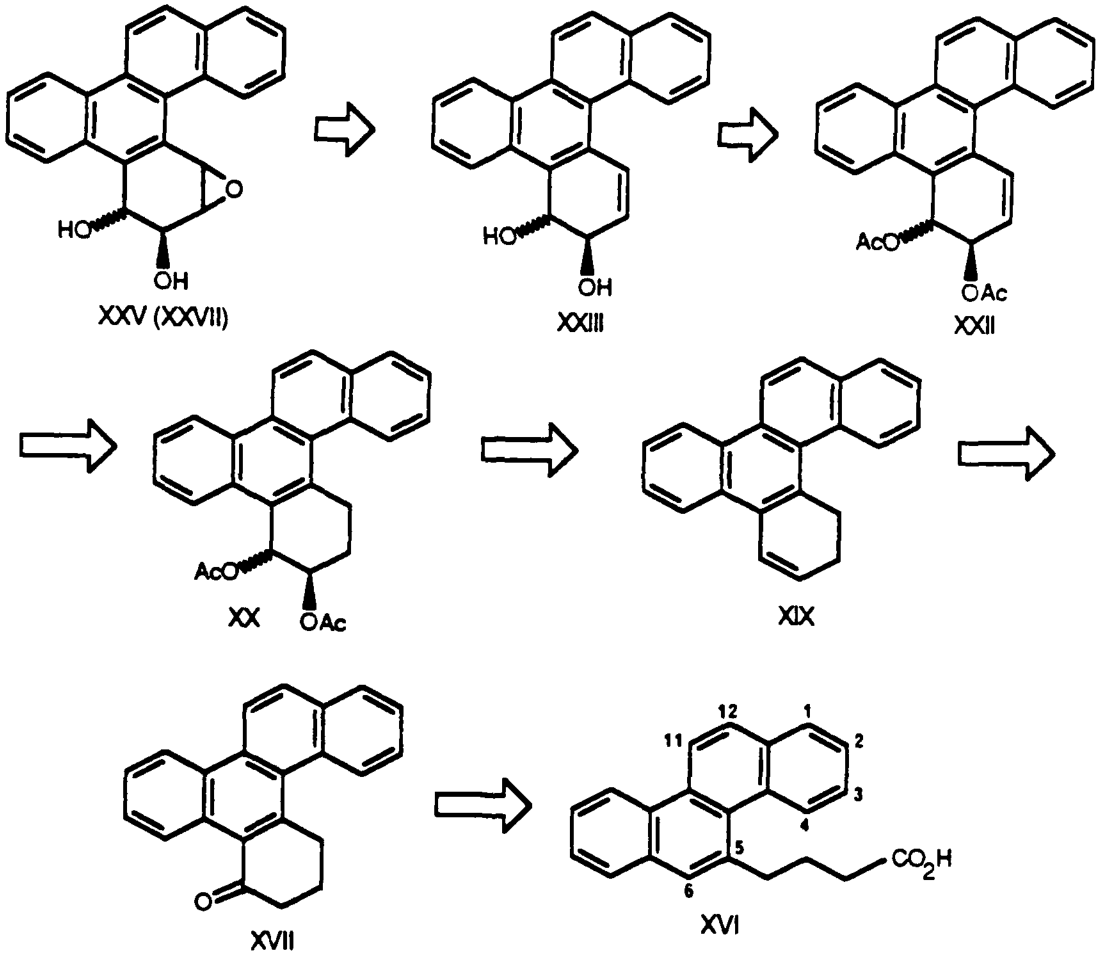
Figure 2.2

1,2,3,4 Benzo Ring Target Molecules



A retrosynthetic scheme is shown in Scheme 2.1.

Scheme 2.1



An obvious precursor to ketone XVII appears to be 4-(5-chrysenyl)-butanoic acid (XVI). However, preparation of this 5-substituted chrysene was a synthetic challenge.

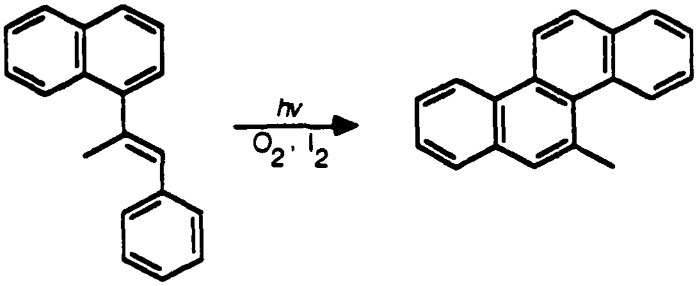
Beyer reported a reaction in 1938 of succinic anhydride with chrysene using aluminum chloride (3). By changing reaction solvent (benzene versus nitrobenzene) he obtained different products. With benzene, he reported C_6 substitution and while using nitrobenzene, he obtained C_5 substitution. Our attempts to repeat the procedure using nitrobenzene did not produce the desired acid (4), as was determined by predicted NMR integration ratio (5). That is, the expected ratio of aromatic protons for 5-substitution of a carbonyl is shown in Table 2.2. These results do not agree with observed NMR spectra. More likely, reaction was occurring at the 1,2, or 6 positions. Carruthers found similar results for chrysene acetylation (6).

Table 2.2 (7,8)

Table of Calculated Substituted Chrysene Proton Chemical Shifts

Substituted	# Protons by Chemical Shift (ppm)		
Position	7.5-8.39	8.4-8.9	9
1	6	4	1
2	6	4	1
3	7	3	1
4	7	4	
5	7	4	
6	6	4	1
Chrysene	8	4	
Observed	6	4	1

After various attempts in benzene, nitrobenzene, and methylene chloride, we determined this route could not directly provide the desired material. In 1977, Nagel reported the synthesis of 5-methylchrysene through photolysis of appropriately substituted stilbenes (9).



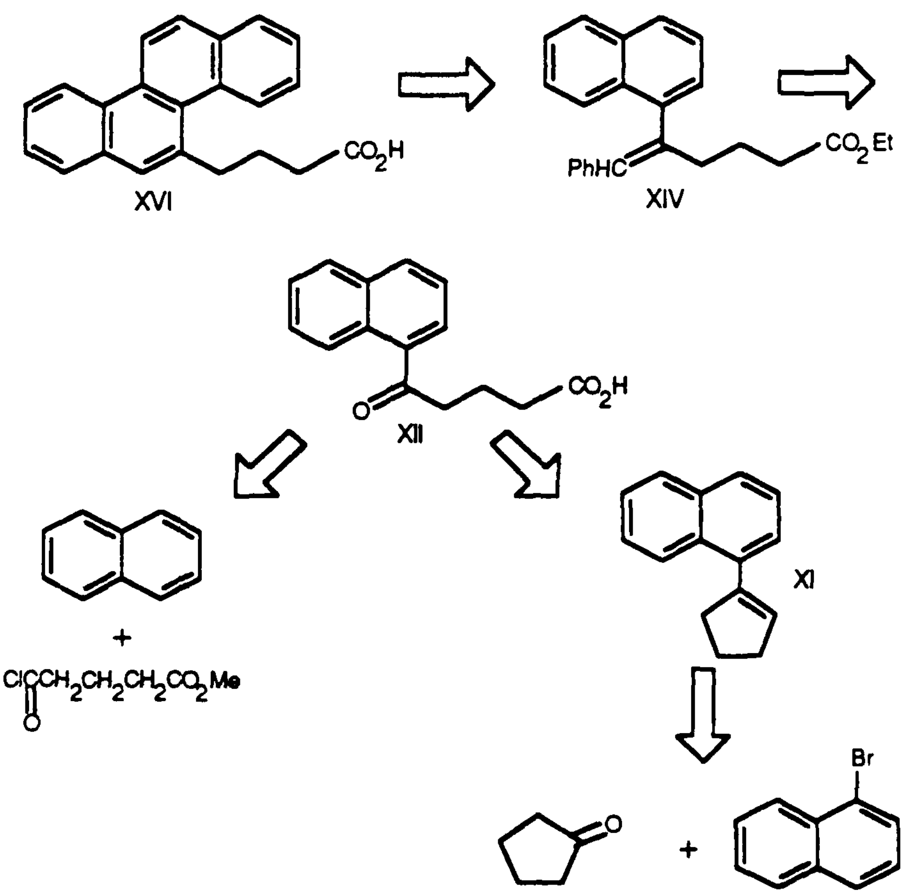
This method appeared feasible if the precursors could be made as shown in Scheme 2.2. This precursor is possible through a Wittig reaction of benzyltriphenyl phosphonium chloride and 5-(1-naphthyl)-5-oxo-glutaric acid (ester).

Two routes to this glutaric acid seem feasible. The first is a direct Friedel-Crafts acylation reaction with naphthalene and methyl 4-(chloroformyl)butyrate. The second method consists of oxidative cleavage of cyclopentene-substituted naphthalene.

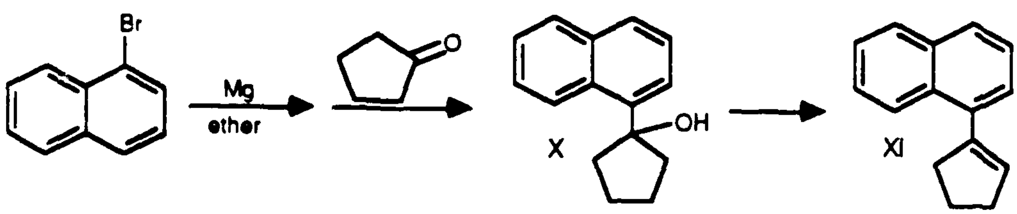
Although method 1 is the most direct, actual preparations resulted in mixtures containing as high as 20% of the 2- substituted product (by NMR of crude product). This appeared to result in difficult to separate mixtures. Our desire to know the precise substitution pattern before carrying on to the photolysis step led us to use method 2.

A Grignard reagent was prepared using 1-bromonaphthalene (10). This reagent was then reacted with cyclopentanone to prepare (1-naphthyl)-1-cyclopentanol. Dehydration of the alcohol was accomplished with refluxing benzene and catalytic *para*-toluene sulfonic acid (11,12).

Scheme 2.2



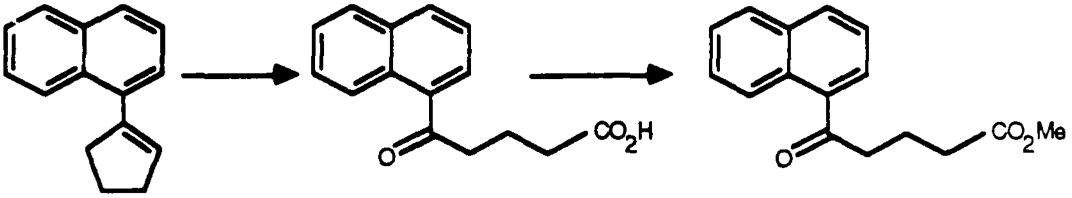
Scheme 2.3



Although it was possible to isolate the alcohol from the Grignard reaction, it was unnecessary and the crude product can be directly dehydrated. However, at the time of purification of (1-naphthyl)-1-cyclopentene, one must exercise precaution if any naphthalene is present. The alkene can be purified by Kugelrohr distillation (120-160°). However, naphthalene will plug the system. Naphthalene should first be removed by Kugelrohr distillation at temperatures below 110°. The overall yield of alkene was 40%. The NMR spectrum of alcohol X is shown in Figure 2.3. The NMR spectrum of alkene XI gave a signal at δ=6.06ppm, characteristic of an alkene absorption (Figure 2.4).

Two methods for the preparation of 5-(1-naphthyl)-5-oxo-glutaric acid (ester) from the cyclopentyl derivatives appeared feasible. The first involved direct oxidative cleavage of the alcohol while the other involved oxidative cleavage of the alkene (13). The results are summarized in Table 2.3. Oxidative cleavage of the alcohol was set aside because of difficulties in purification and removing residual color.

Scheme 2.4



As illustrated in Table 2.3, there were a number of variations attempted using $KMnO_4$ (14,15). In general, all of those tried gave the desired keto acid; however, as the results show, not all were practical for larger scale reactions. In particular reaction 2 provided a respectable 63% yield when working on 100mg scale, but dropped dramatically to 14% on the multi-gram scale. A similar trend was noted in reactions 3 and 4. However, on larger scale, changing the reaction solvent from benzene to tetrahydrofuran provided yields of approximately 30% (reaction 5).

Table 2.3

Formation of 5-(1-Naphthyl)-5-Oxo-Glutaric Acid

Rxn	Starting Material	Reagents	g sm./g acid produced	% yield
1	X	CrO_3/HOAc*	2.00/0.723	31.7
			4.00/1.537	33.7
			4.00/0.550	12.1
2	XI	$KMnO_4$/18-crown-6/C_6H_6	.157/.124	63.6
			1.00/.175	14.1
			10.20g/.752	5.9
			1.00g/.670	54.0
			8.541g/1.50g	14.2
			Average yield	=12.4
3	XI	$KMnO_4/H_2O$/acetone/0°	1.00/.560	45.1
			5.20/1.738	26.9
			1.00/.513	41.3
			4.074/.8286	16.4
			4.074/1.245g	24.6
			Average yield	=25.6

Table 2.3 (cont.)

Formation of 5-(1-Naphthyl)-5-Oxo-Glutaric Acid

Rxn	Starting Material	Reagents	g sm./g acid produced	% yield
4	XI	$KMnO_4/NaIO_4$	.618/.319	41.6
			6.18/1.204	15.7
			Average yield	=18.1
5	XI	$KMnO_4$/18-crown-6/THF	.500/.197	31.8
			5.00/1.617	26.1
			3.87/2.687	56.0
			12.0/7.00	47.0
			5.00/1.764	28.4
			12.0/5.918	39.7
			12.0/3.932	26.4
			23.3/7.154	24.7
			10.353/4.781	37.2
			Average yield	=33.6

*Chromium oxidation gave "colored" products.

In every case, unreacted alkene remained in the reaction. It appears that as the manganese dioxide forms, it coats unreacted permanganate and stops the reaction progress. Addition of a large excess of permanganate only resulted in the formation of over oxidized products in larger quantities. No effort was made to dry the THF, hence water may also increase the solvent's ability to dissolve the permanganate and make it more available for reaction before becoming coated with MnO_2.

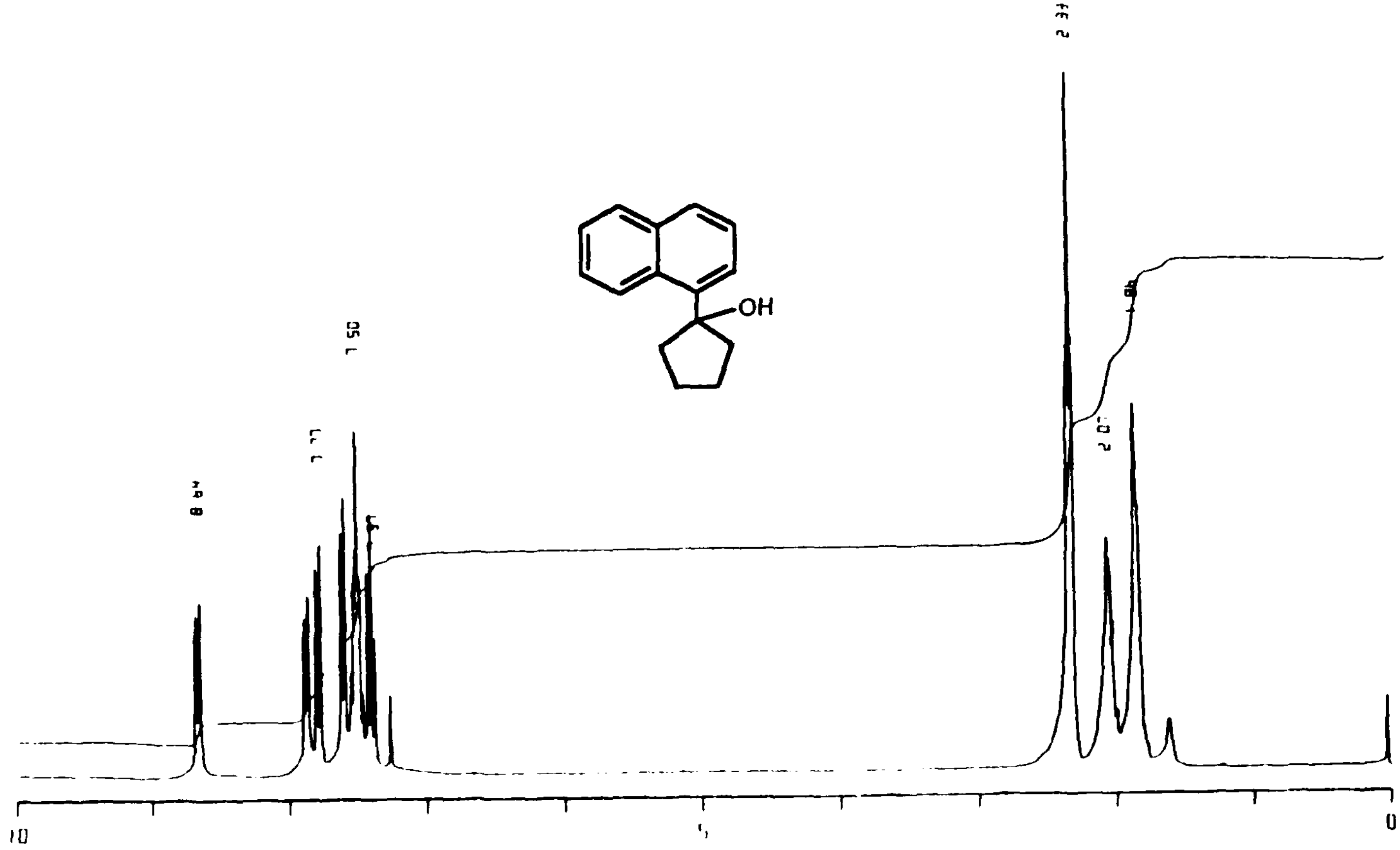


Figure 2.3 300 MHz NMR Spectrum ($CDCl_3$) of X

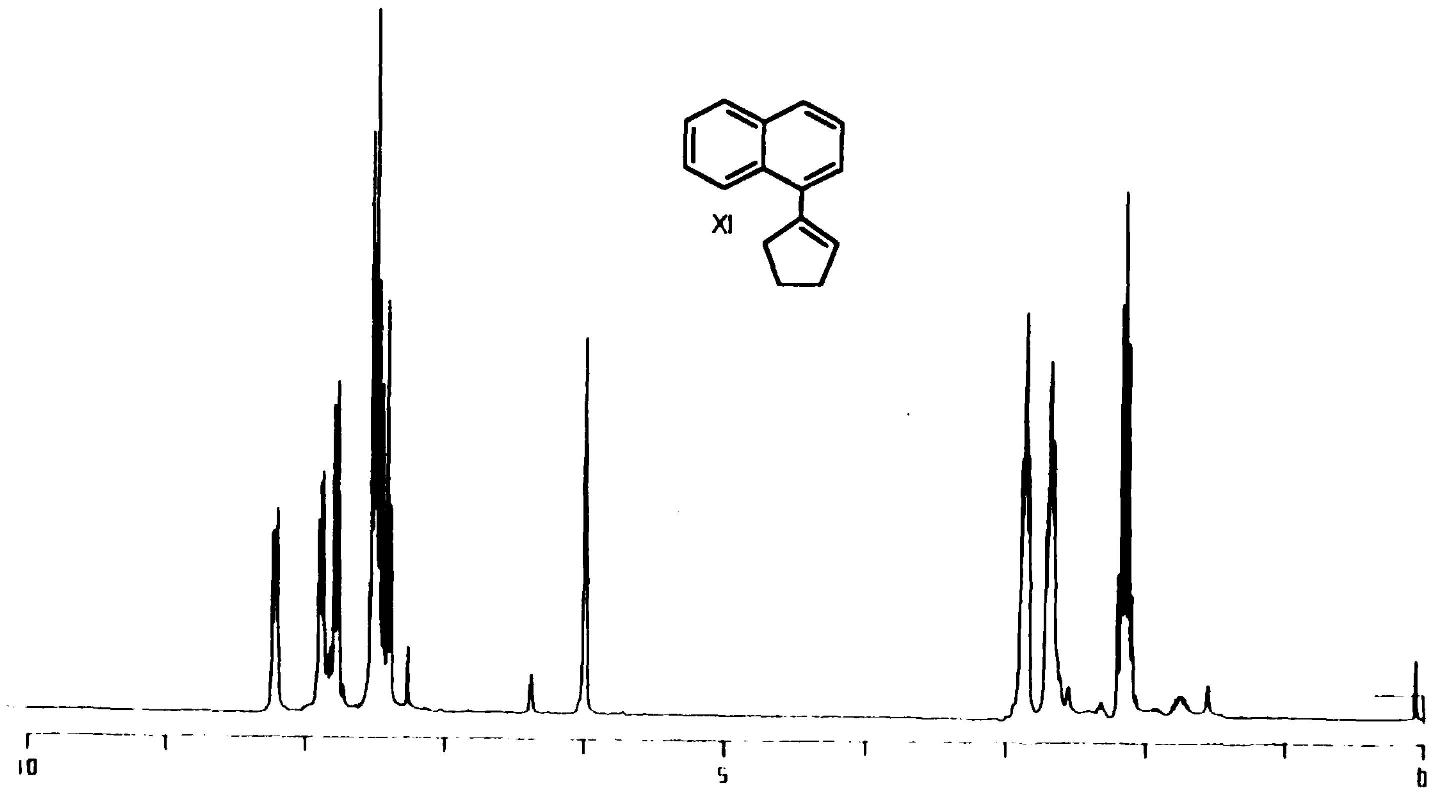


Figure 2.4 300 MHz NMR Spectrum ($CDCl_3$) of XI

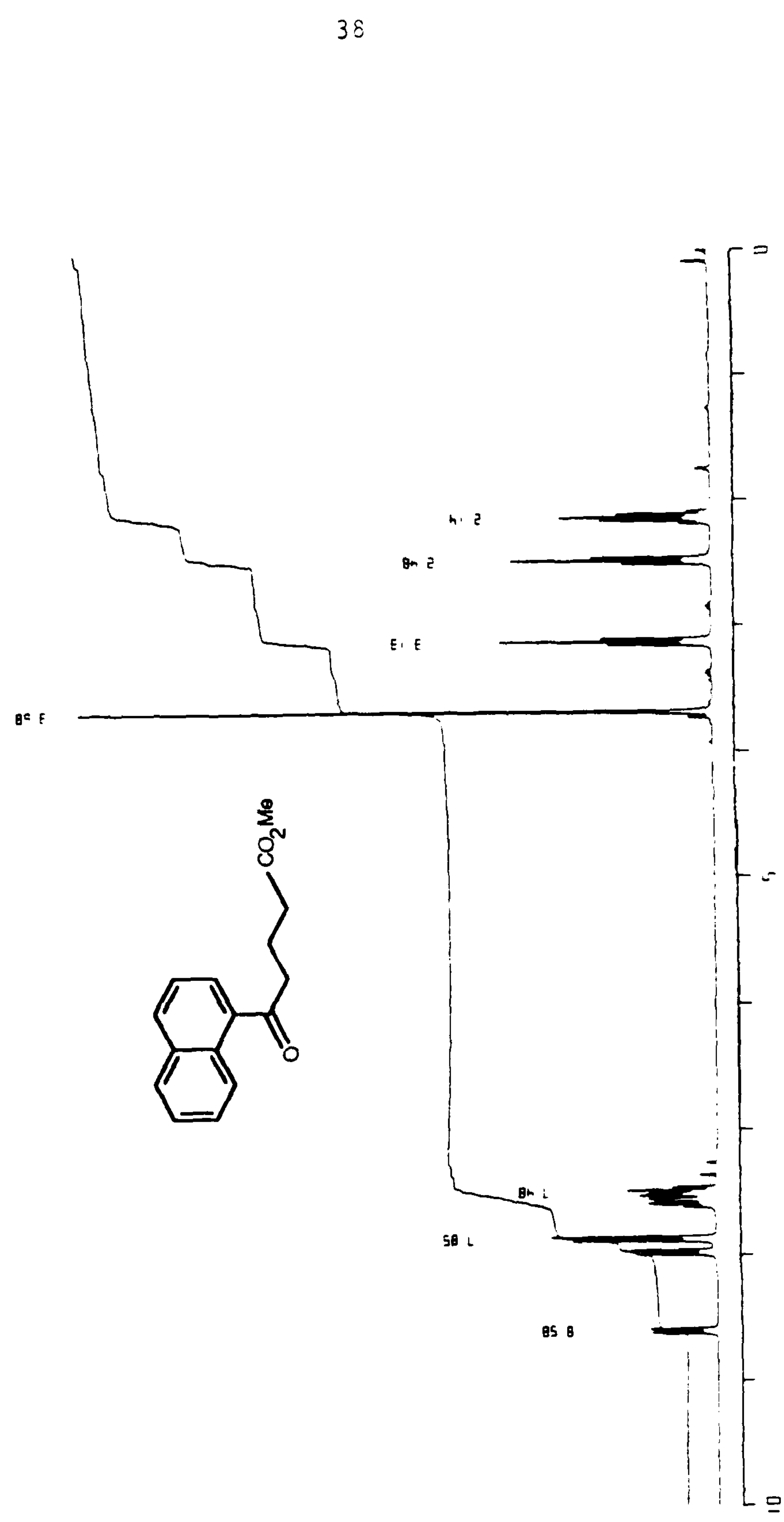


Figure 2.5 300 MHz NMR Spectrum ($CDCl_3$) of XIII

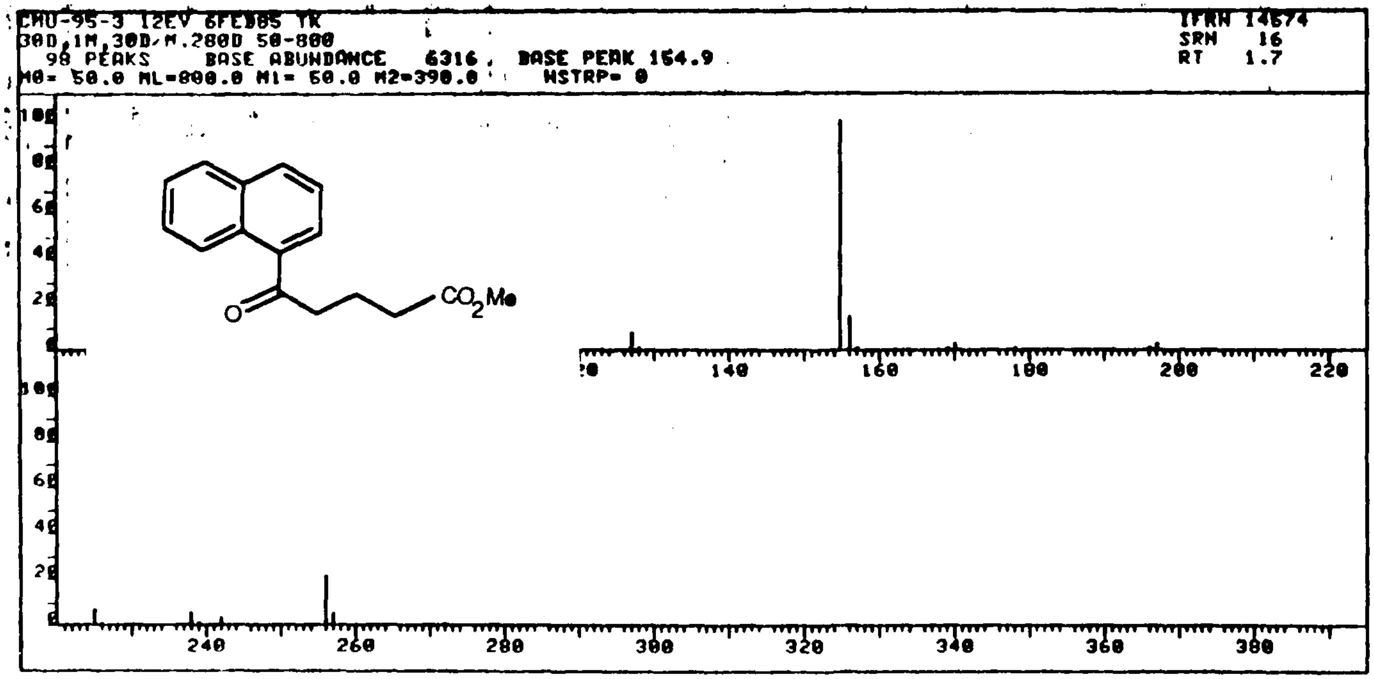


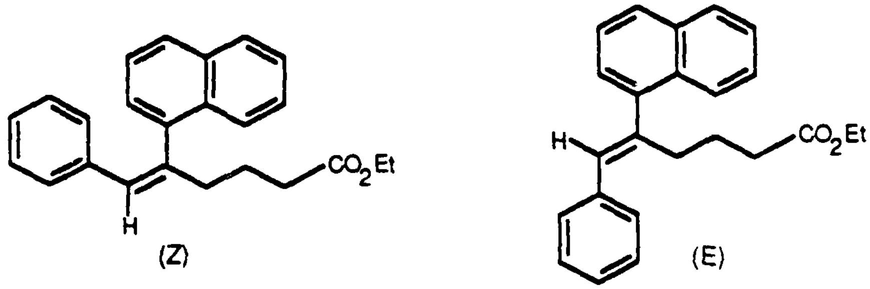
Figure 2.6 12 eV Mass Spectrum of XIII

The keto acid formed from the oxidative cleavage readily formed the methyl ester by reflux in acidic methanol (see Figures 2.5 and 2.6). This protection step was necessary to avoid destruction of the Wittig reagent in the next step.

The Wittig reagent was prepared by the reaction of sodium ethoxide and benzyltriphenyl phosphonium chloride. Attempts to use methanol and dimethylsulfoxide as solvents were unsuccessful and starting material was recovered. By using ethanol (16), dried using magnesium ethoxide, the reaction proceeded and was complete after 36 hours of reflux. Reaction progress was monitored by thin layer chromatography with 4% ethyl acetate in benzene as the developing solvent. Reaction product could be separated from the reaction salts by first evaporating the ethanol, dissolving the residue in dichloromethane and washing with water. Concentration of material by flash chromatographic technique with benzene provides both the E and Z isomer. No effort was made to separate the two isomers since both are satisfactory

Figure 2.7

E and Z Stilbene Isomers

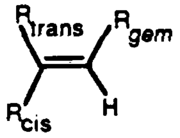


precursors for photolysis. However, by examining the proton NMR spectra of the mixture, two distinct alkene singlets are apparent.

The absorption at δ=6.62 was assigned to the Z isomer while that of δ=6.75 given to the E isomer. One could predict that the E isomer should be farther downfield based on the proximity of the benzylic hydrogen to two aromatic substituents while in the Z isomer the proton is somewhat distant from the naphthyl substituent. Calculations predicting the chemical shift of these protons also agree.

Table 2.4

Chemical Shift Calculation (7)



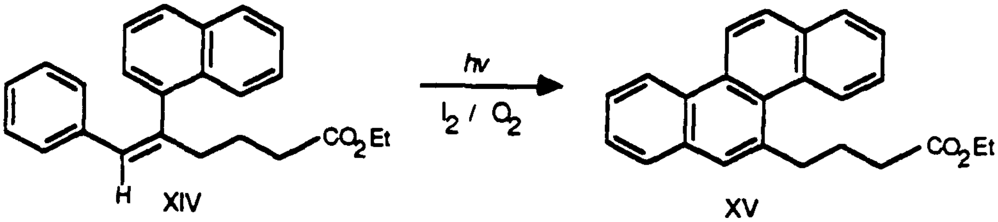
$$\delta = 5.28 + Z_{trans} + Z_{cis} + Z_{gem}$$

A. For Z isomer $\delta = 5.28 + (-.10) + (-.26) + 1.35 = 6.27$

B. For E isomer $\delta = 5.28 + (-.29) + .37 + 1.35 = 6.71$

More evidence for this assignment comes from photochemical reaction of the mixture. One would predict that the Z isomer should react at a faster rate since it is already in the proper geometry. The E isomer must first isomerize to the Z before photochemical cyclization can occur. Reaction progress was followed by NMR. The fact that the absorption at δ = 6.62 initially decreases faster than that at 6.75 is consistent with the alkene proton assignments.

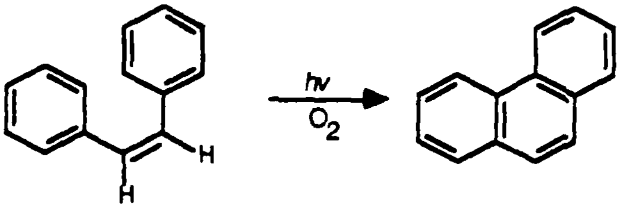
Scheme 2.5



XIV XV

The next step in the synthesis involves the photocyclization of the stilbene analogue. Cis-stilbene is known to form phenanthrene through photolysis in the presence of an oxidant (17). This type of reaction appears general with stilbenes unless substitution patterns or the carbon backbone structure sterically hampers the reaction (16).

Scheme 2.6

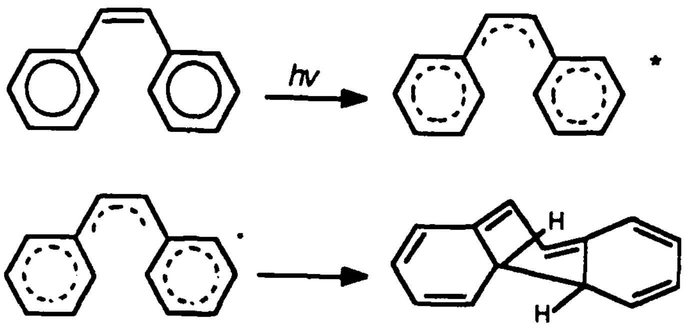


Photocyclization proceeds first by absorption of a photon followed by ring closure to give a dihydro aromatic. These dihydro compounds are normally unstable and are either oxidized to the aromatic ring system, or ring open to starting material. Normally oxygen or iodine is used as oxidizing reagent.

The current view of the mechanism has been recently reviewed (16). This is shown in Figure 2.8.

Figure 2.8 (16)

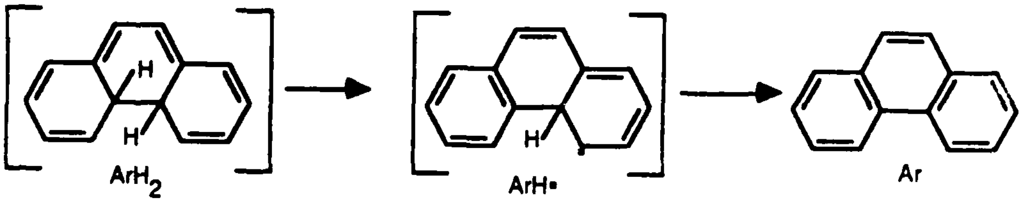
Mechanism of Photocyclization



When the reaction is carried out in an air-saturated solution, or with small amounts of iodine, the dihydro intermediate is converted on to the aromatic compound.

Figure 2.9 (16)

Conversion to Aromatic System

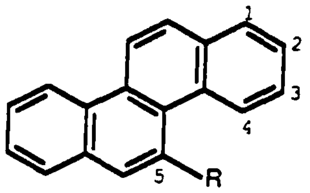


Initiation: $I_2 + h\nu \longrightarrow 2I^{\bullet}$

Propagation: $I^{\bullet} + ArH_2 \longrightarrow ArH^{\bullet} + HI$

$ArH^{\bullet} + I_2 \longrightarrow Ar + HI + I^{\bullet}$

There are a few examples in the literature for preparing five-substituted chrysenes by photolysis; however, none have dealt with more than two-carbon side chains (3, 18, 19).



Initial attempts at ring closure failed. In all cases, the reaction was carried out in cyclohexane with iodine. However, initial reactions were carried out at 350nm using reagent grade cyclohexane. An ultraviolet spectrum of the naphthylstyrene indicated absorption at 280nm and none at 350nm. In addition, a spectrum of reagent grade cyclohexane indicated the presence of impurities with strong uv absorption from 190 through 280nm. Subsequent reactions were carried out at 300nm using cyclohexane that was first stirred over concentrated sulfuric acid, then washed with water, and distilled.

Table 2.5

Conversion of Naphthyl Styrene to Chrysene

g stilbene	Vol C_6H_{12}	gI_2	reactant conc(m)	I_2 conc (g/1)	Rxn time	% conversion by NMR*
50mg	50ml	trace	.0029		48hr	N.R.
100mg	100ml	trace	.0029		52hr	81.5
834mg	300ml	30mg	.0081	.1	28hr	33.0
751mg	300ml	30mg	.0073	.1	72hr	--
400mg	500ml	160mg	.0023	.32	24hr	--
828mg	600ml	260mg	.0030	.325	16hr	83.3

Table 2.5 (cont.)

Conversion of Naphthyl Styrene to Chrysene

g stilbene	Vol C_6H_{12}	gI_2	reactant conc(m)	I_2 conc (g/l)	Rxn time	% conversion by NMR*
2.500g	11	260mg	.0073	.26	96hr	91.8
1.00g	11	260mg	.0029	.26	48hr	69.4
1.00g	11	260mg	.0029	.26	24hr	80.3
1.767	11	400mg	.0051	.40	20hr	48.7
1.581g	11	358mg	.0046	.36	20hr	47.5
1.500g	11	350mg	.0044	.35	20hr	80.9
1.500g	11	100mg	.0044	.10	60hr	72.3
1.500g	11	90mg	.0044	.090	72hr	96.4
1.552g	1.51	100mg	.0044	.067	96hr	73.9
1.622g	1.51	80mg	.0047	.053	72hr	69.6

*Measured by comparing loss of absorption at $\delta=2.82$ and appearance of absorption at $\delta=3.63$ ppm.

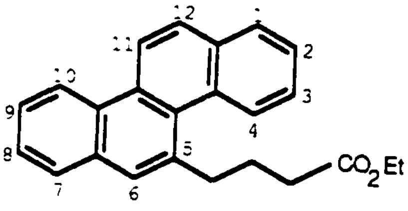
More reproducible results, and good yields, were obtained when the iodine concentration was less than or equal to .1g/l. Apparently an increase in this concentration reduces the amount of light that can enter into reaction with the naphthyl styrene. Additionally, we found that larger concentrations of iodine caused a film to form on the reaction vessel surface, again decreasing the reaction efficiency.

Photolysis of the naphthyl styrene (1.5-1.6g) was carried out for 72-96 hours in cyclohexane with 80-100mg of iodine while stirring and bubbling dry air through the system. Thin layer chromatography indicated one major compound, origin material, and several minor components. Analysis of the worked up mixture by NMR indicated that 77% of the recovered material was the desired chrysene (or 53% yield overall, since more polar material was removed in the workup procedure).

Table 2.6

Proton Chemical Shifts and Coupling Constants for

4-(5-Chrysenyl)-Ethylbutyrate (Aromatic Region)



XV

Proton	PPM	multiplicity	coupling constants (Hz)
1	7.99	dd	$J_{1,2}=7.6$, $J_{1,3}=1.8$
2,3,8,9	7.65	m	
4	8.82	dd	$J_{3,4}=9.2$, $J_{2,4}=1.6$
6	7.88	s	
7	7.91	dd	$J_{7,8}=7.6$, $J_{7,9}=2.4$
10	8.69	d	$J_{9,10}=7.0$
11	8.71	d	$J_{11,12}=9.1$
12	7.96	d	$J_{11,12}=9.1$

The structure of the 5-substituted chrysene was verified by proton nuclear magnetic resonance and comparison with reported literature values of related compounds. Observed data for the 300MHz NMR spectrum are shown in Table 2.6.

Table 2.7

Proton Chemical Shift for 5-Methylchrysene (20)

proton	ppm	multiplicity
1,7	7.95	m
2,3,8,9	7.63	m
4	9.00	m
6	7.87	s
10	8.78	m
11	8.77	d
	($J_{11,12}=9$)	
12	7.98	d

Table 2.8

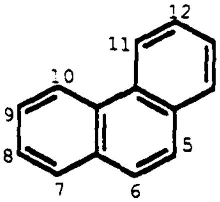
Proton Chemical Shift for Chrysene (8)

proton	ppm
1,7	8.00
2,8	7.64
3,9	7.72
4,10	8.79
5,11	8.73
6,12	8.02

NMR spectra of related compounds from the literature are provided (Tables 2.7-2.9) for comparison. Proton assignments were based on these literature comparisons and coupling values found in XV. In particular H_4, now in a hindered region, displays a downfield shift when compared to

Table 2.9

Partial NMR Spectrum For Phenanthrene and 9-n-Propylphenanthrene* (21)



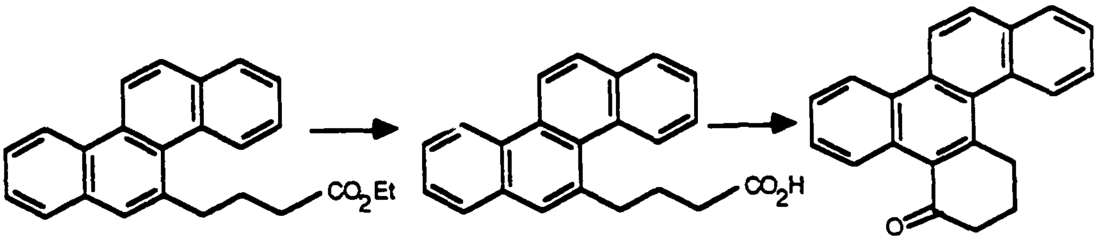
Proton	Phenanthrene (ppm)	9-n-propyl phenanthrene (B)
7	7.81	7.65 $J_{7,8}=7.9$, $J_{7,9}=1.8$, $J_{7,10}=0.5$
8	7.48	7.40 $J_{7,8}=7.9$, $J_{8,9}=7.0$, $J_{8,10}=0.8$
9	7.55	7.43 $J_{8,9}=7.0$, $J_{7,9}=1.8$, $J_{9,10}=9.2$
10	8.62	8.46 $J_{9,10}=9.2$, $J_{8,10}=0.8$, $J_{7,10}=0.5$
6	7.64	7.41
11	8.62	8.54 $J_{11,12}=8.8$, $J_{11,13}=1.0$, $J_{11,14}=0.5$

*phenanthrene renumbered for ease of comparison with chrysene.

chrysene. This is also consistent with 5-methyl chrysene and normally attributed to "edge deshielding" (5). The singlet at 7.88ppm was assigned to H_6. There is a slight upfield shift for this proton. However, it is quite consistent with assignments for 5-methyl chrysene and changes in shift values relative to the corresponding unsubstituted hydrocarbon, e.g., -0.14ppm for XV, -0.15ppm for 5-methyl chrysene and -0.23ppm for 9-n-propylphenanthrene.

Rigorous purification of the ethyl ester wasn't attempted. Instead, the ester was hydrolyzed to the acid by reflux in 10% KOH/water in 76% yield. The acid was crystallized from methanol and the crystals were washed with diethyl ether and dried. The mass spectrum gave a molecular ion m/e 314. NMR data agree with the structure assignment. (See Figures 2.10 and 2.11.)

Scheme 2.7



XV XVI XVII

Purification at this point was necessary since cyclization as shown in Scheme 2.7 did not provide readily separable product mixtures when crude starting materials were used. A variety of conditions and reagents were examined. These are summarized in Table 2.10 (22).

Ketone XVII was purified by flash chromatography with benzene as the solvent. The product was easily crystallized by adding a small amount of ethyl acetate and cooling. The mass spectrum provided a molecular ion (296) and major peaks

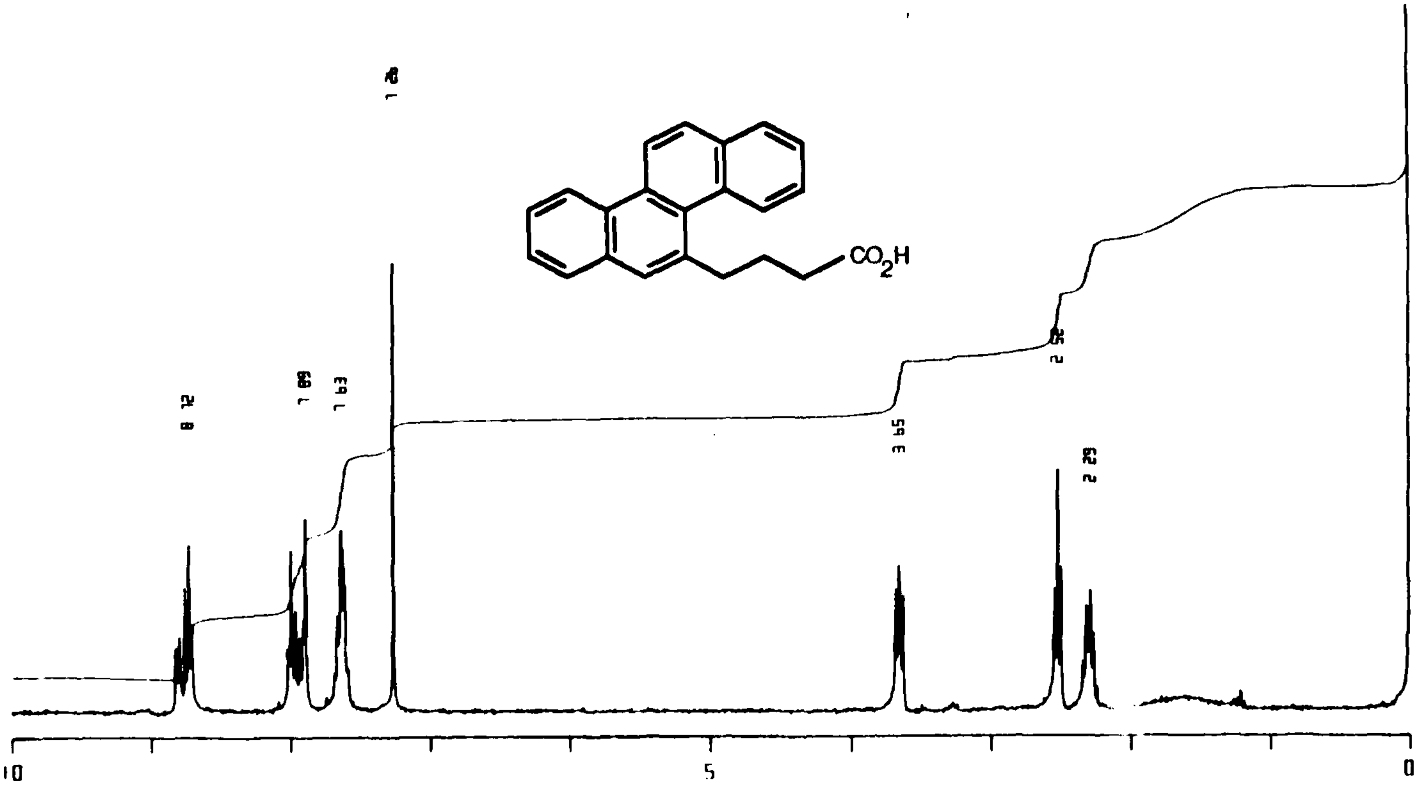


Figure 2.10 300 MHz NMR Spectrum ($CDCl_3$) of XVI

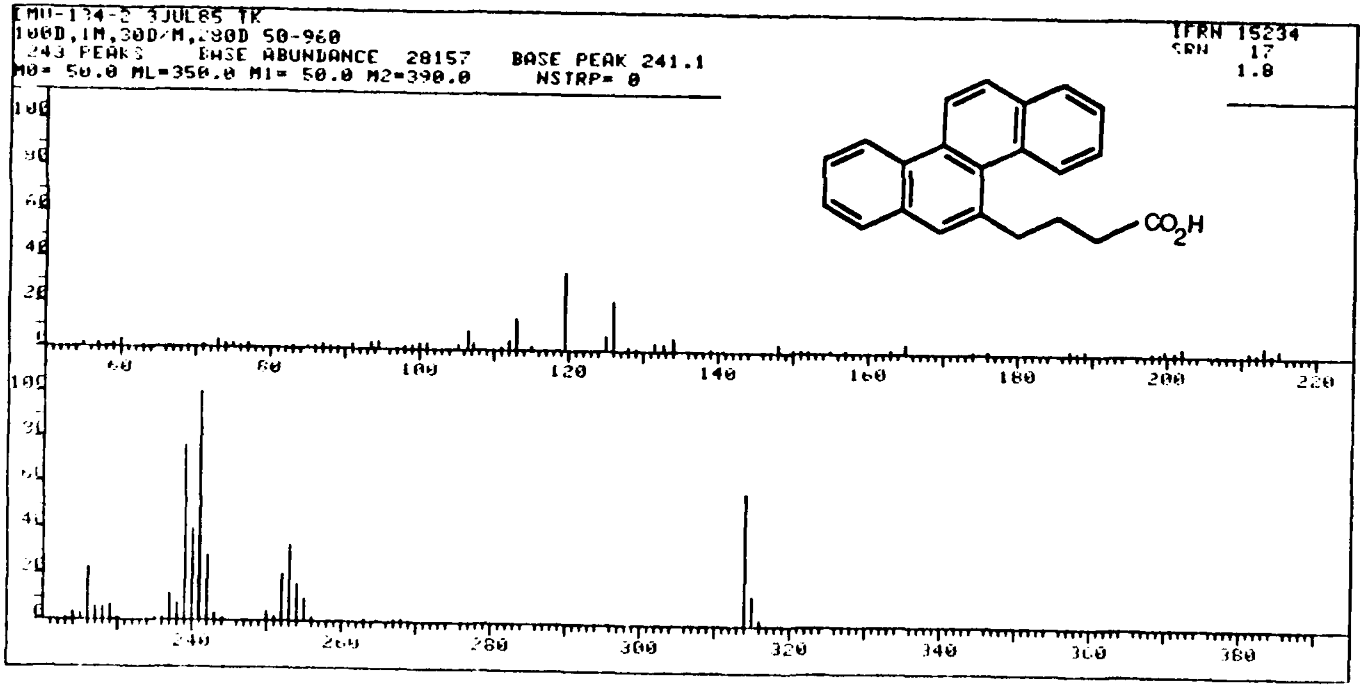


Figure 2.11 70 eV Mass Spectrum of XVI

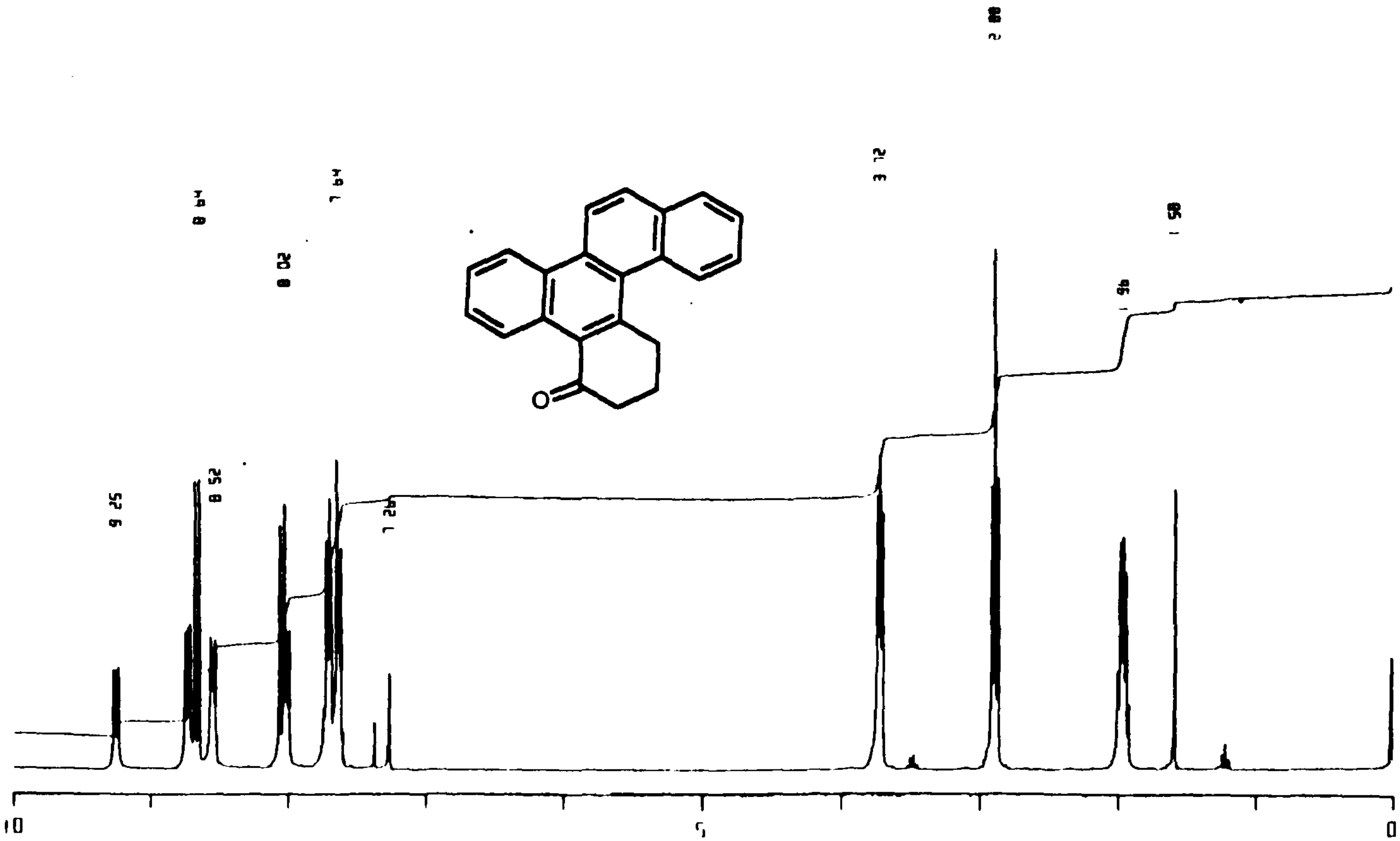


Figure 2.12 300 MHz NMR Spectrum ($CDCl_3$) of XVII

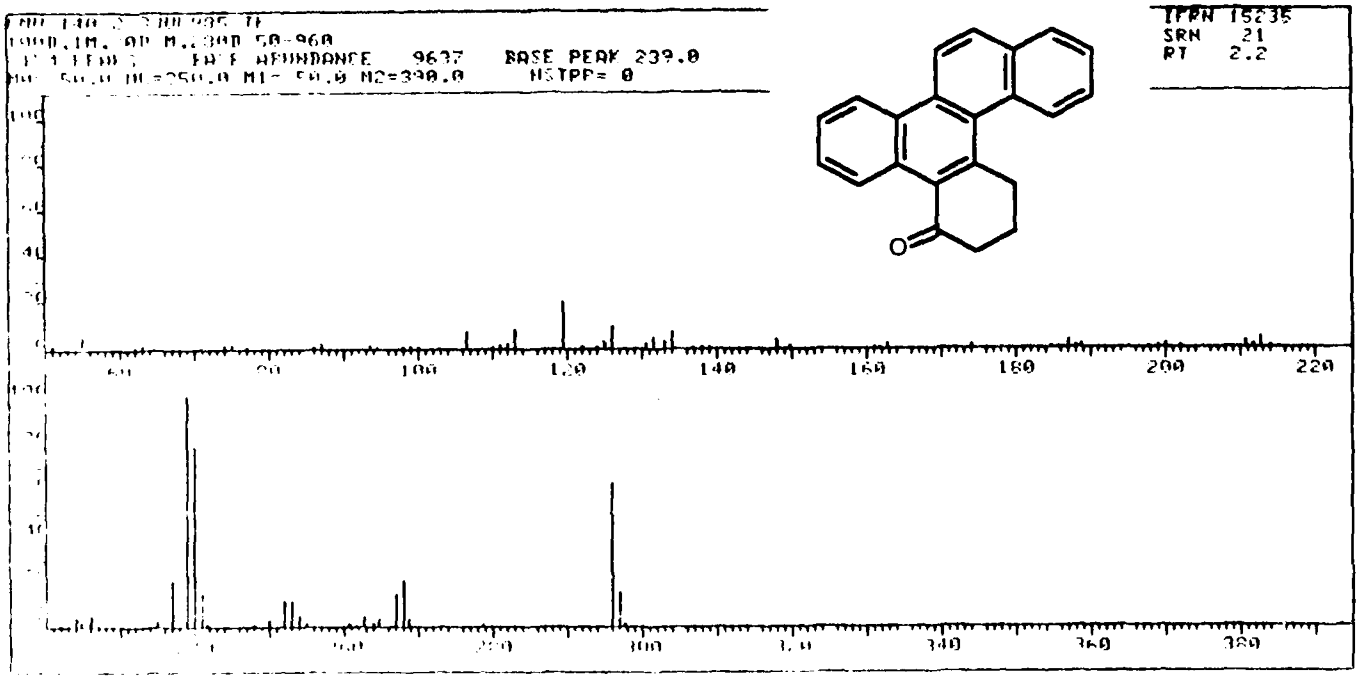


Figure 2.13 70 eV Mass Spectrum of XVII

Table 2.10

Synthesis of 1,2-dihydrobenzo(g)chrysene-4(3H)-one

Method	XV	Reagent	Rxn time	%yield
1	Et ester	10%CH_3SO_3H/CH_2Cl_2/R.T.	16hr	N.R.
2	Et ester	10%CH_3SO_3H/CH_2Cl_2/reflux	8hr	N.R.
3	Et ester	10%P_2O_5/CH_3SO_3H/80°	1hr	31.2
4	acid	10%P_2O_5/CH_3SO_3H/50°	1hr	47.7
5	acid	10%CH_3SO_3H/CH_2Cl_2/R.T.	96hr	58.1
6	acid	CH_3SO_3H/70°	1hr	86.4
7	acid	CH_3SO_3H/55°	2.5hr	84.8

at 268 (20.3%), (loss of CO or C_2H_4), 240 (loss of CO or C_2H_4), and 239 (loss of C_2H_5 from 268). NMR verified the structure by giving distinct aromatic absorptions at δ=9.25ppm (m, H_9) and δ=8.64ppm (d,J=9.1,H_{13}) 8.02 (d,J=9.1,H_{14}). Individual multiplets accounting for H_4, H_{12} and H_1 (8.72, 8.52, and 7.99) contained meta couplings. The multiplet at 7.64 was attributed to H_2, H_3, H_{10}, and H_{11}. An authentic sample of triphenylene ketone was available and gave reasonable NMR comparison. (See Figure 2.14.)

Figure 2.14

NMR Data for Triphenylene Ketone

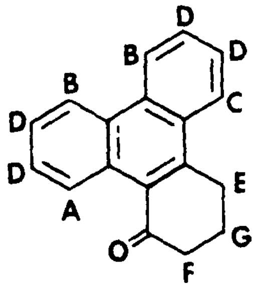
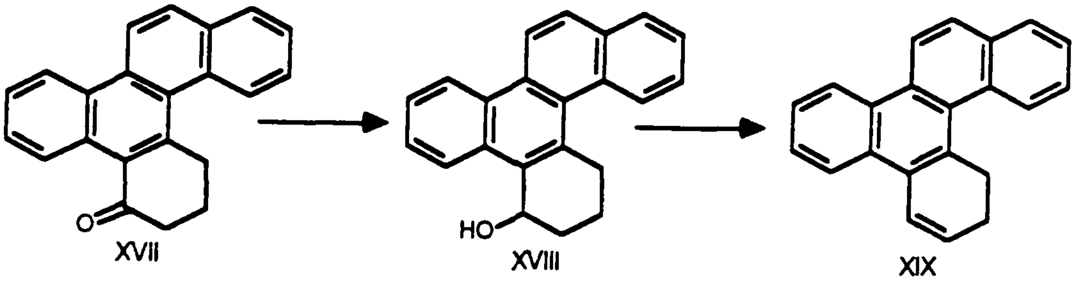


Figure 2.14 (continued)

Proton	(ppm)	Proton	(ppm)
A(m)	9.24	D(m)	7.66
B(m)	8.66-.69	E(t)	3.43
C(d,J=8.2)	8.19	F(t)	2.84
		G(p)	2.31

Scheme 2.8



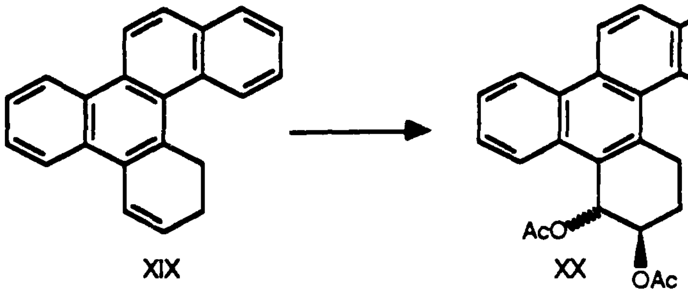
Ketone XVII was easily reduced at room temperature to the alcohol XVIII using sodium borohydride in methanol. A four-fold excess of $NaBH_4$ was used and the reaction went to completion in one hour. Progress was followed by thin layer chromatography with benzene as the solvent.

This reaction routinely gave 96-99% yield of crude product. A broadened singlet at δ=5.77ppm was assigned to the carbinol proton.

Carbinol XVIII, without further purification, was dissolved in benzene. A few small crystals of para-toluene sulfonic acid were added and the mixture was stirred overnight. Reaction progress was again followed by thin layer chromatography. Purification was by flash chromatography with petroleum ether to give 85.4% yield of alkene XIX from the ketone. NMR data is shown in Figure 2.15 and the mass spectrum is shown in Figure 2.16.

Metabolites derived from polycyclic aromatic hydrocarbons are known to have a trans-vicinal-dihydroxyl configuration. One of the more efficient methods to introduce this functionality is the Prévost reaction (23), in which trans-diacetates are introduced initially, and are then hydrolyzed to the diol. This method has been used in the synthesis of several PAH metabolites.

Scheme 2.9



The Prévost reaction was run with silver acetate and iodine in benzene. For reaction success it is imperative that all reagents and solvents be dry. Silver acetate and I_2 were stirred together in dried benzene. The reaction vessel was covered with aluminum foil. When the red iodine - benzene color faded - usually 15-30 minutes - alkene XIX was added in one batch and stirring was continued at room temperature for one hour. The mixture was then brought to reflux for 3.5 hours. The diacetate XX was purified by flash chromatography using first benzene and then 5% ethyl acetate in benzene to elute the diacetate is 80-90% yield.

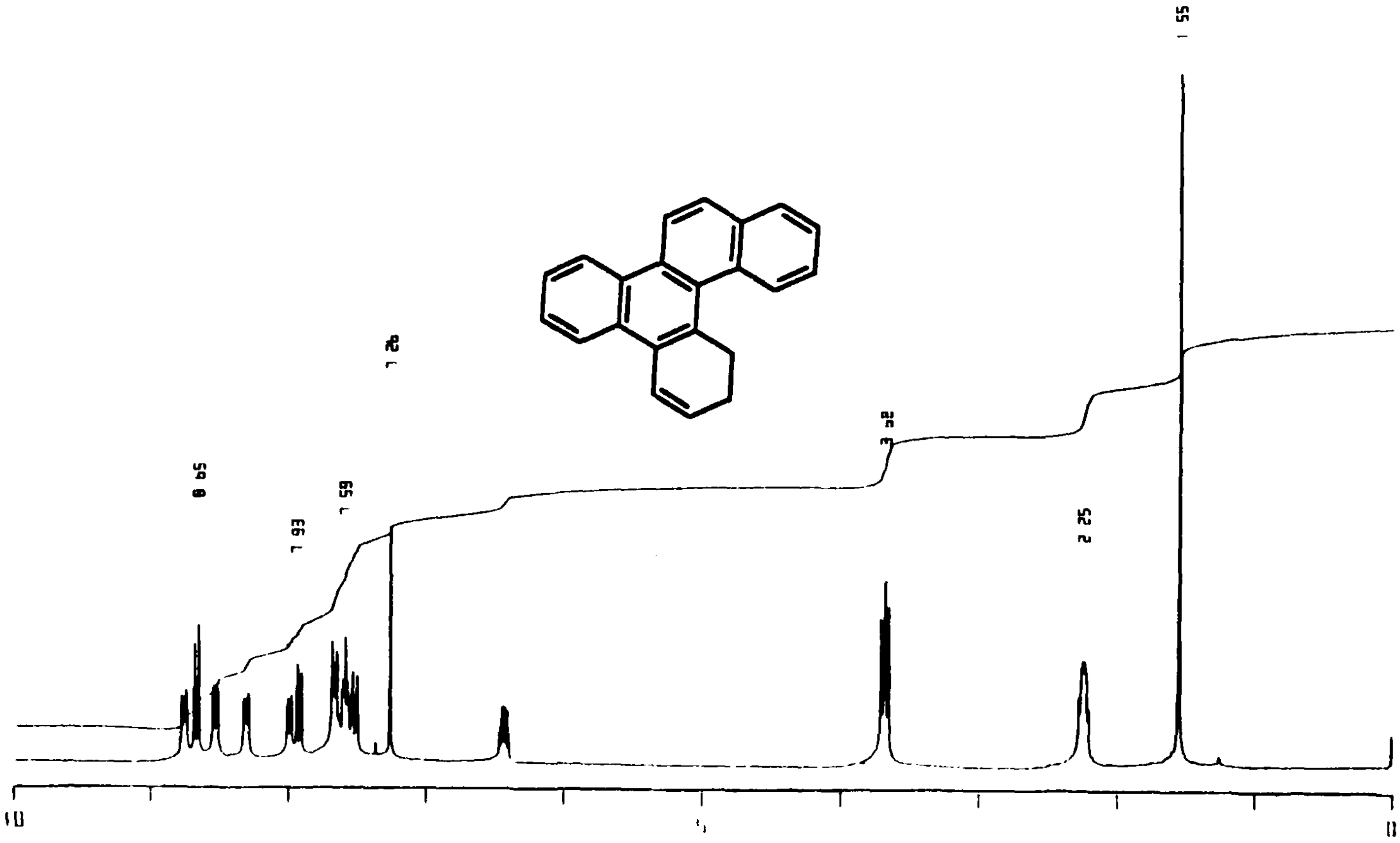


Figure 2.15 300 MHz NMR Spectrum ($CDCl_3$) of XIX

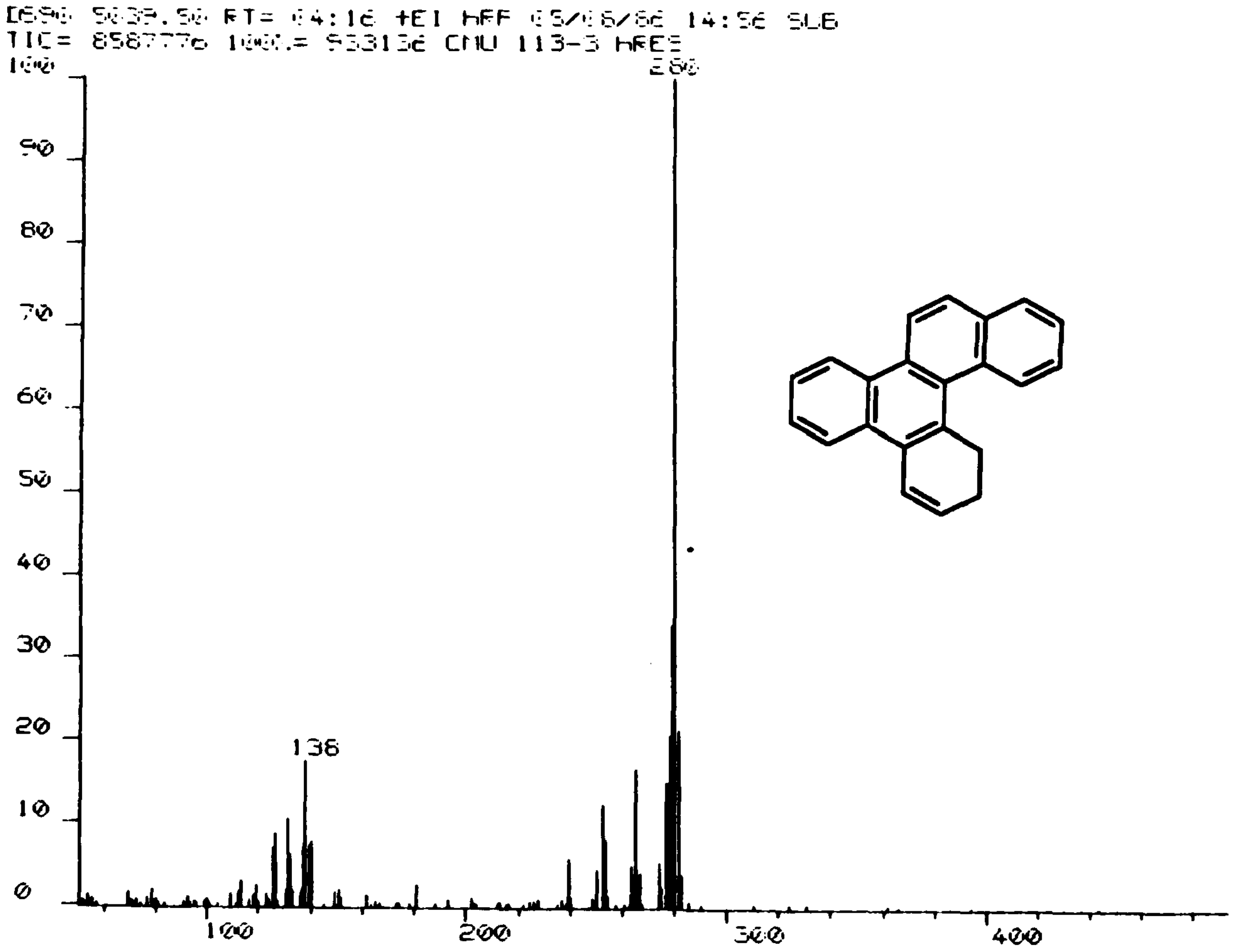
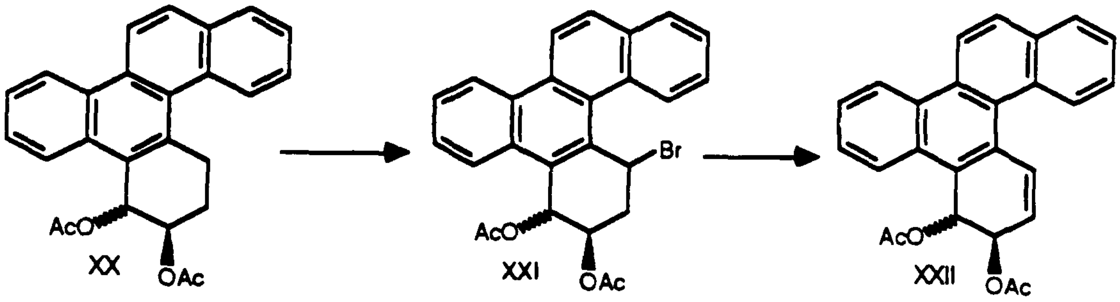


Figure 2.16 High Resolution Mass Spectrum of XIX

Mass spectral results (Figure 2.16) indicated that the diacetate had indeed formed and provided a molecular ion of 398 with fragments at M^+-60 (338, -$C_2H_4O_2$) and M^+-120 (278,-2x$C_2H_4O_2$). NMR (Figure 2.17) further verified the structure, with acetate absorptions at δ=2.06 and 2.19ppm. Other NMR data: 8.6-8.77(m,3H), 7.9-8.0(m,3H), 7.6-7.7(m,4H), 6.84(d,3.1Hz,1H), 5.48(m,1H), 3.6-3.9(m,2H), 2.0-2.17(m,2H-in addition to OAc). Of particular interest is the benzylic acetate proton at δ=6.84ppm. The coupling constant for this proton, 3Hz, is typical for a vicinal diequatorial relationship of the hydrogen atoms, implying that the acetates are in a quasidiaxial conformation. (Note: vicinal diaxial proton coupling would be expected to be 8-10 Hz.) An axial-equatorial relationship was eliminated based on the chemical route, since the Prévost conditions supply *trans* products.

Scheme 2.10



Our attempts at preparing bromo diacetate XXI initially yielded inconsistent results. In general the reaction was done using N-bromosuccinimide (NBS) in carbon tetrachloride (CCl_4) and heating to 55-70°. Initially, azobisisobutyronitrile (AIBN) was used as a radical initiator. Only a catalytic amount (tip of spatula) of AIBN was used. The reaction mixture was saturated with N_2 gas and N_2 was bubbled through the mixture for the entire reaction time. After 25-35 minutes at 55-60° a fine precipitate formed in the reaction vessel. The temperature was maintained at 60° for an additional 5 minutes and the flask was cooled. The precipitate was filtered and the solvent was evaporated. Attempts to purify at this stage by column chromatography or by crystallization were fruitless, and resulted in increasing decomposition. The crude material was carried on to the dihydro diacetate *XXII* by reaction of 1,5-diazabicyclo [4.3.0]non-5-ene (*DBN*) in *THF* at -10°. Dihydro diacetate XXII was confirmed as the product and will be discussed later.

Attempts to repeat this series of reactions met with failure. Bromination reactions of XX with NBS and AIBN no longer gave material that would dehydrobrominate to XXII. Reactions were run over a 40° to 70°C temperature range. To systematically reduce errors,a variety of experiments were run on *XX* and are summarized in Table 2.11.

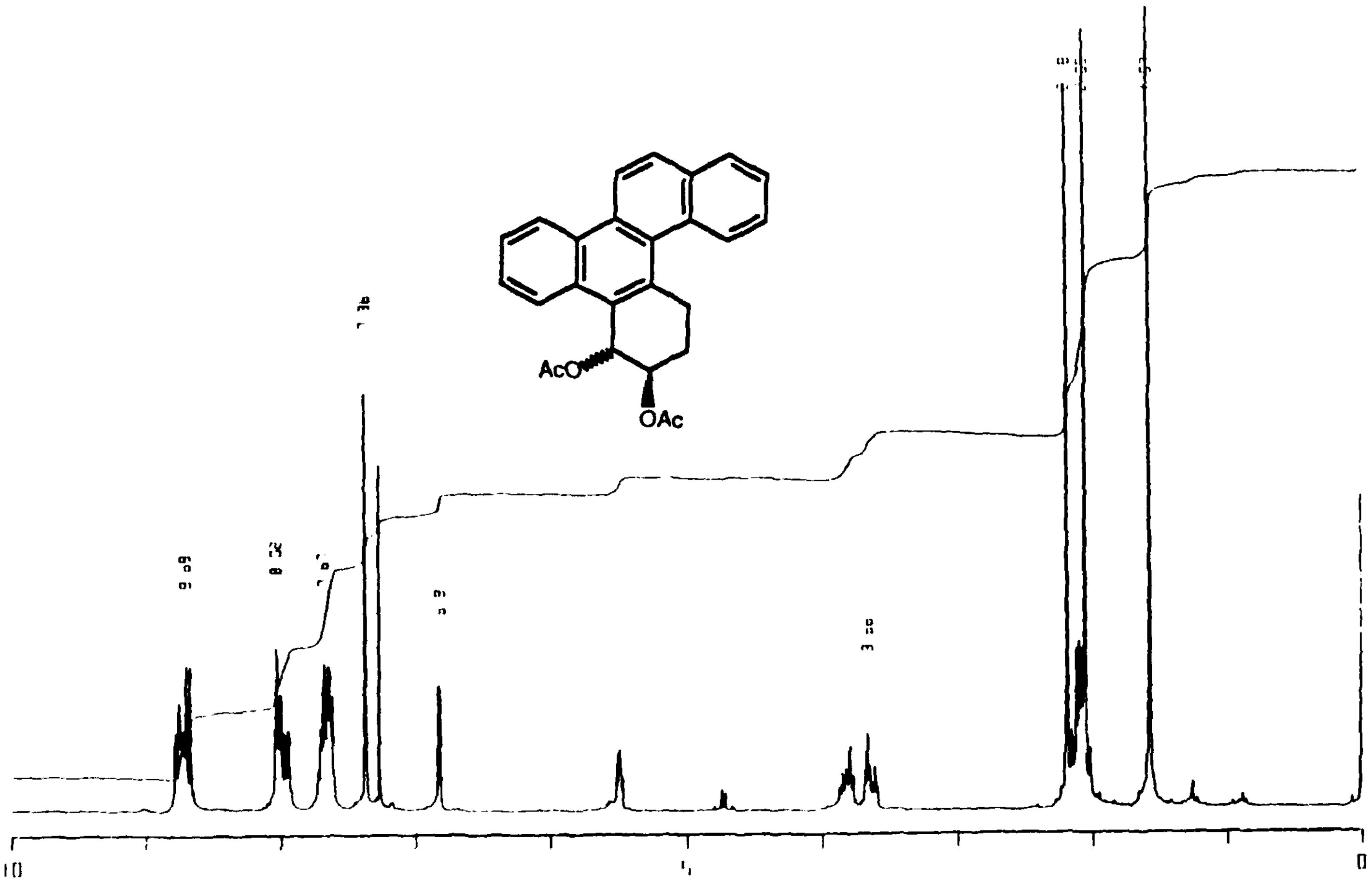


Figure 2.17 300 MHz NMR Spectrum ($CDCl_3$) of XX

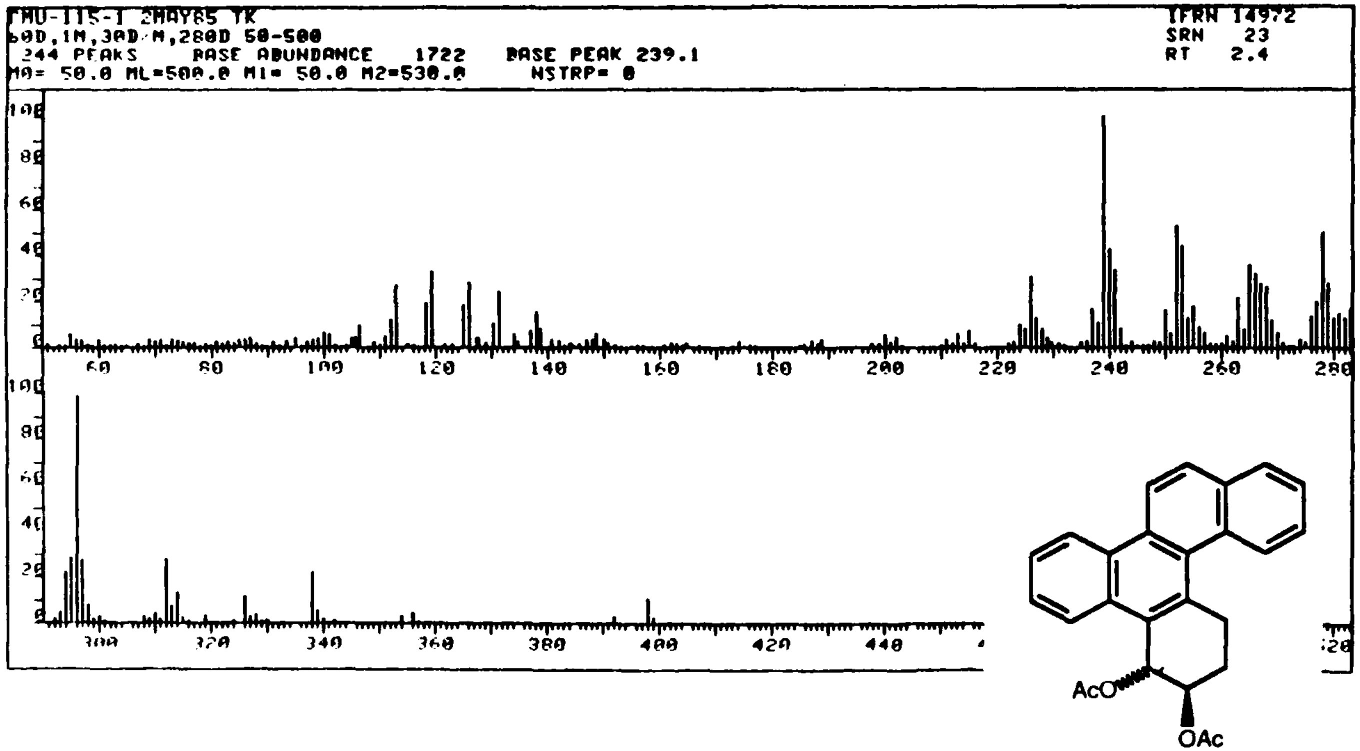


Figure 2.18 70 eV Mass Spectrum of XX

Table 2.11

Bromination of XX

$$XX \longrightarrow XXI$$

Reagent(s)	Temperature	Results
none	50°	No decomp
$NaHCO_3$	50°	No decomp
$NBS/NaHCO_3$	50-55°	bromination
NBS	50°	unknown product

We discovered that the bromination product, XXI, is sensitive to any acid that might be present in the reaction medium (e.g. HBr) or even residues on the reaction glassware. Since XX was reasonably stable at the reaction temperature, it seemed logical to add solid base to the medium. In addition, all glassware, stir bars, and cintered glass funnels were washed with dilute ammonium hydroxide and distilled water prior to drying. The reaction was carried out by adding a large excess of solid $NaHCO_3$ to the flask containing tetrahydro diacetate (XX) and NBS. After heating to 55° for 30-35 minutes bromination occurred, but it was still impossible to isolate XXI and, in fact, XXI wasn't the major product. By examining the NMR spectra, we found that XXI was very susceptible to dehydrobromination under thermal conditions. Dihydro diacetate XXII was actually the major product. To avoid unwanted thermal decomposition of XXII, the reaction temperature was maintained at 60° in an oil bath. Dehydrobromination was complete at 180 hours.

Identical product was obtained when the bromination reaction was stopped at 2.5 hours, and this mixture containing both XXI and XXII was reacted with DBN overnight at -10°.

The reaction series XX → XXI → XXII presented another interesting problem due to formation of a phenolic derivative (see Table 2.12). At times this became a major contaminant. Initially, we believed this product formed during the dehydrobromination step due to excessive DBN. Our initial solution was to remove the impurity. Since DBN formed an ammonium salt of the phenol, it remained at the origin during a rapid filtration through silica gel with THF as solvent. This filtration was the first step of the workup of the DBN reaction. A second procedure carried the impurity on through subsequent acetate hydrolysis, after which it was removed.

Table 2.12

Preparation of Dihydrodioldiacetate

Reaction	Bromination Method	Dehydrobromination Method	Product
1	A	E	1+2
2	A	E	1+2
3	A	E	1+2*
4	A	E	1+2*
5	A**	-	
6	A**	-	
7	A**	-	
8	A**	-	

Table 2.12 (continued)

9	C	E	1
10	C	E	1+2***
11	B	D	1+2****
12	B	D	1
13	B	D	1
14	B	D	1
15	B	D	1
16	B	E	1
17	B	E	1

*filtered through silica gel with THF, **Br-product not observed, ***Observed by TLC, ****Not observed originally but on work up.

Method A XX $\xrightarrow[CCl_4]{NBS/AIBN}$

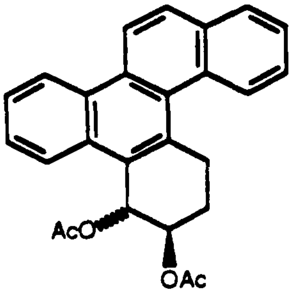
B XX $\xrightarrow[CCl_4]{NBS/NaHCO_3}$

Method C XX $\xrightarrow[CCl_4]{NBS}$

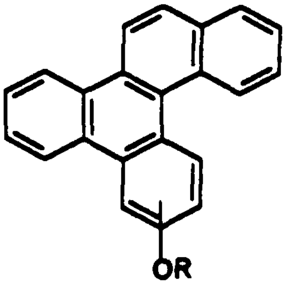
D XXI $\xrightarrow[\Delta]{}$

E XXI $\xrightarrow[DBN]{}$

Product 1



Product 2



However, an even better result was noted in reactions 9 through 17. In reaction 9, all glassware was cleaned and rinsed with dilute NH_4OH before drying. As a result, product 2 wasn't observed. During the work up of reaction 11, a cintered glass filter that hadn't been treated with NH_4OH was used to remove the residue succinimide. As a result, product 2 was seen on TLC after the work up but not before filtration. Reactions 12 through 17 were not plagued by this impurity, possibly due to the reaction medium containing $NaHCO_3$ through all stages. It appears that any source of acid throughout this sequence had devastating effects as were particularly noted in reactions 5 through 8. Without any base treatment of glassware or readily available base in the reaction, dihydro diacetate (XXII) wasn't produced consistently and yields of brominated compound (XXI) were reduced, based on NMR analysis.

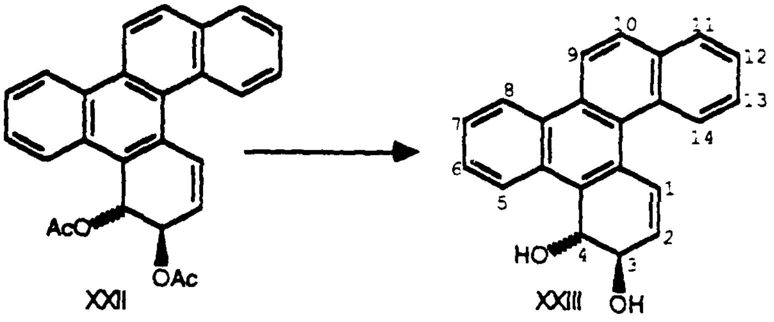
An additional experiment was run to determine the effect of DBN on XXII. Dihydro diacetate (XXII) in THF was reacted with DBN under the same conditions as used in the dehydrobromination step. No additional reaction was observed after 16 hours at -10° by TLC.

As a result, the phenolic derivative formed during this reaction sequence does not form upon addition of DBN and must be produced during bromination. Although the mechanism was not studied, one could envision protonation of the acetate XX followed by loss of acetic acid. Bromination at the benzylic position could occur either before or after loss of acetic acid, but when dehydrobromination occurred one is left with a phenolic acetate. Presumably, DBN deacetylates the phenolic acetate to yield the phenol.

NMR data (Figure 2.19) for dihydrodioldiacetate (XXII) indicates 11 protons in the region 7.2 - 8.8ppm. Decoupling experiments were used to identify H_1. NMR assignments are: δ=8.78(d, J=7.4Hz, H_{14}), 8.67(d, J=9.0Hz, H_9), 8.48(m, H_8), 8.0-8.13(m,3H), 7.66(d, J=9.7, H_1), 7.62-7.72(m, 4H), 6.90(bs, H_4), 6.47(dd, J=9.7, 5.95, H_2), 5.49(dd, J=5.95, 1.7, H_3). The mass spectrum (Figure 2.20) of this compound showed a molecular ion at m/e 396, loss of acetic acid (396-60=336), and loss of ketene (336-42=294).

Deprotection of XXII was done by stirring in ammonia saturated anhydrous methanol solution for 24 hours. Evaporation of the methanol at room temperature was followed by purification with column chromatography. Completely aromatized material was easily removed using 18-20% ethyl acetate in benzene as the solvent. Dihydro diol XXIII was obtained in 82% yield with 30% ethyl acetate in benzene.

Scheme 2.11



XXII

XXIII

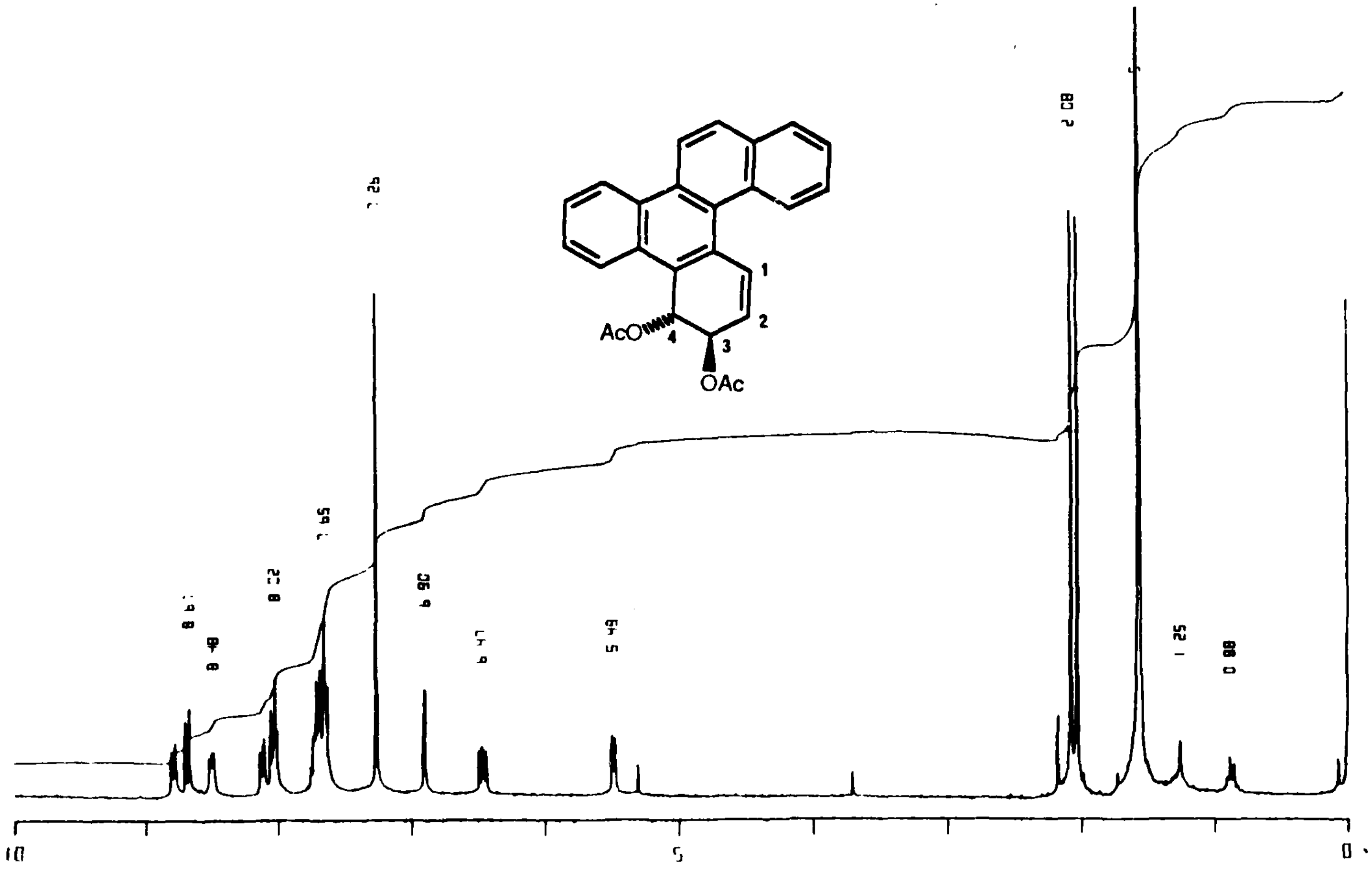


Figure 2.19 300 MHz NMR Spectrum ($CDCl_3$) of XXII

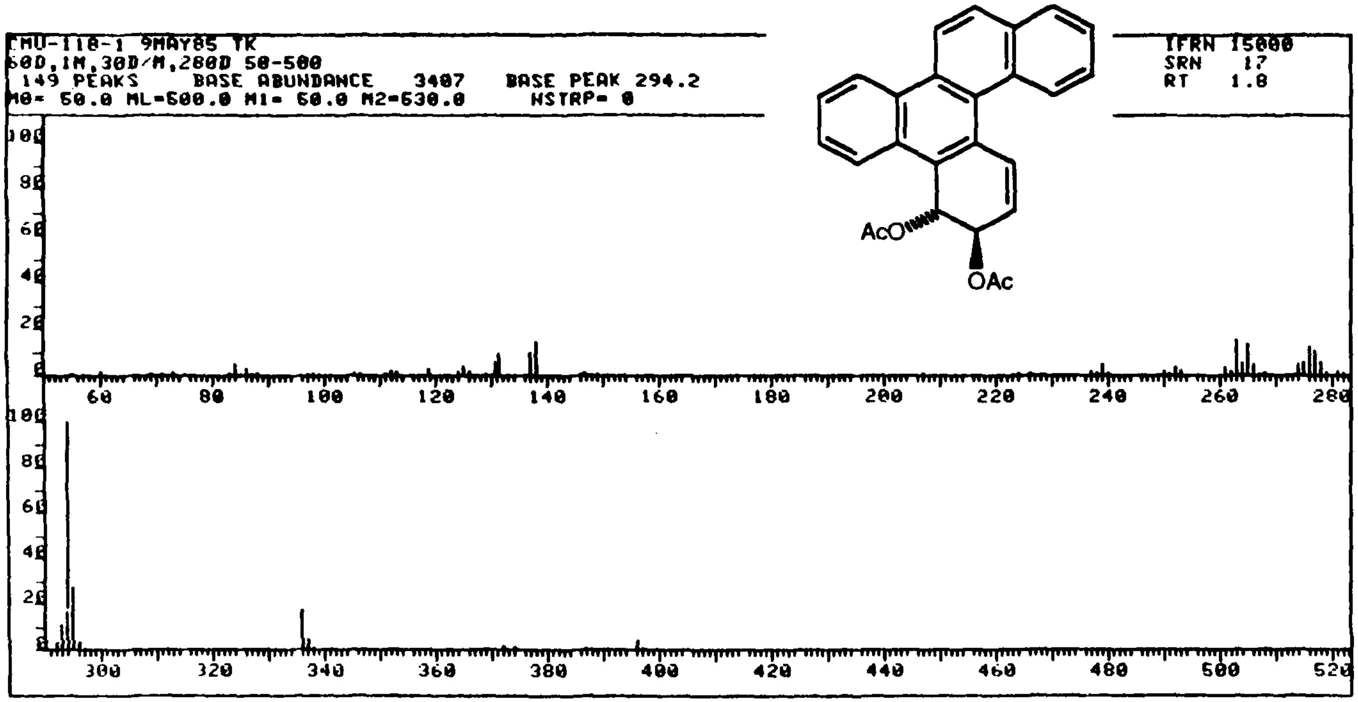


Figure 2.20 70 eV Mass Spectrum of XXII

NMR data: δ =8.77(m, 1H), 8.67(d, J=8.9Hz, 1H), 8.53(m, 1H), 8.47(m, 1H), 8.03(m, 2H), 7.73(m, 2H), 7.63(m, 2H), 7.59 (d, J=9.7Hz, H_1), 6.51(dd, J=9.7, 5.9Hz, H_2), 5.60(dd, J=7.3, 1.8Hz, H_4), 4.69(m, H_3), 1.89(d, J=7.3Hz, 1H(OH)), 1.70(d, J=7.3Hz, 1H(OH)). See Figures 2.21 and 2.22.

The 70eV mass spectrum gave a molecular ion of 312, loss of 18(H_2O) and loss of 28(CO) to give a base peak at 266. Elemental analysis were: calculated for C (84.59%), H (5.16%), O (10.25%), found C (84.50%) and H (5.49%).

Preparation of diol epoxides have been made in the past by using two methods. Route one consists of making an intermediate bromo triol and then eliminating HBr to form the epoxide. The product thus formed is usually the epoxide that is on the same benzo ring face as the benzylic carbinol proton (cis, series 1). The second method is direct epoxidation of the dihydrodiol using peroxyacids. One normally obtains the epoxide on the opposite face of the benzo ring, e.g., trans (series 2) to the benzylic carbinol proton (23). In the synthesis of diol epoxides XXV and XXVII these generalizations were only partially accurate.

Synthesis of bromotriol XXIV was achieved by using bromoacetamide (NBA) in aqueous THF and a catalytic amount of acid. Early attempts to synthesize XXIV proved unsuccessful, producing a bad mixture of products. In these attempts, 10% mole excess of NBA and one drop of concentrated HCl were used in 4 ml of solvent. No attempts to rigorously isolate and purify the mixture were made.

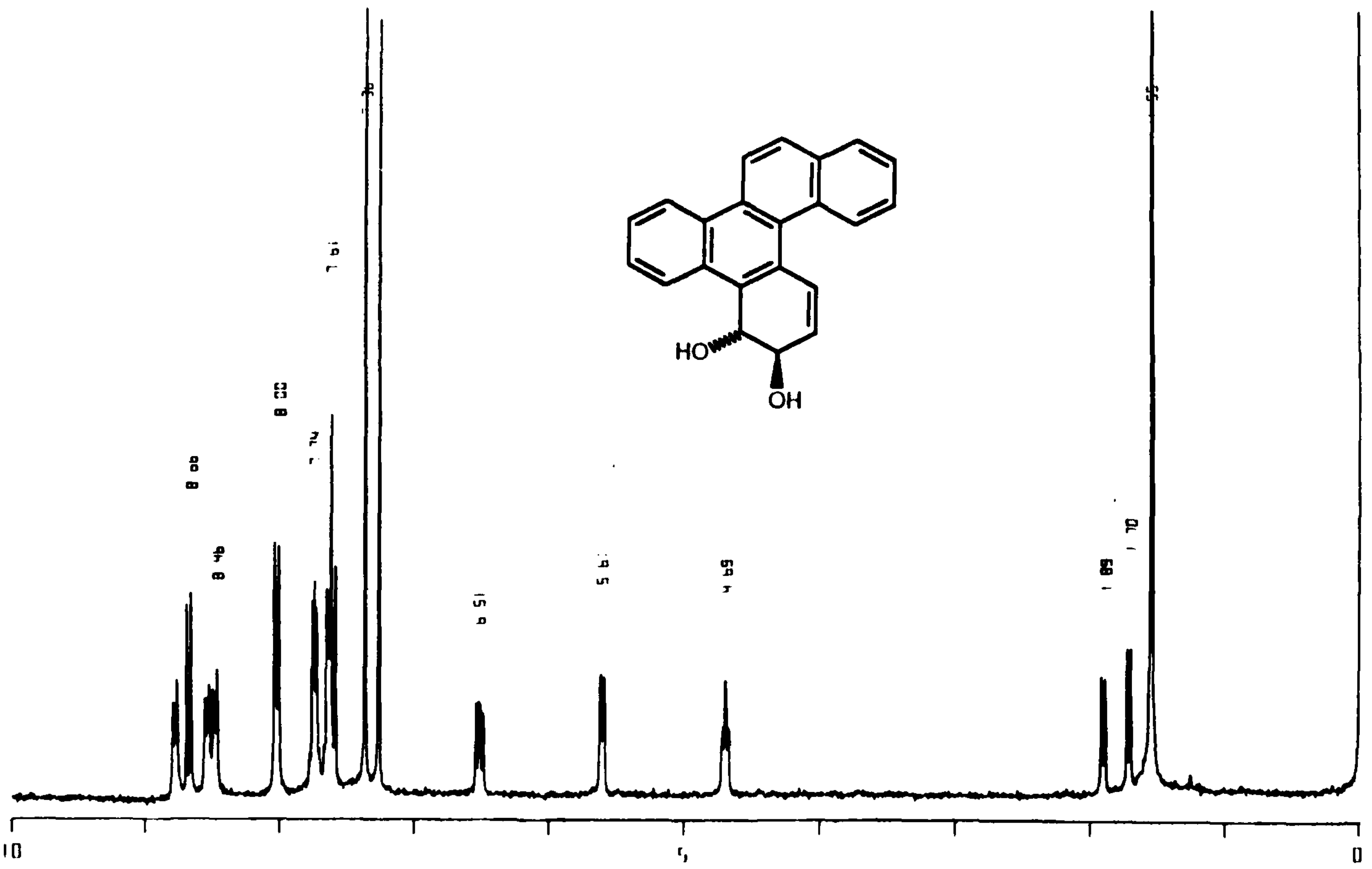


Figure 2.21 300 MHz NMR Spectrum ($CDCl_3$) of XXIII

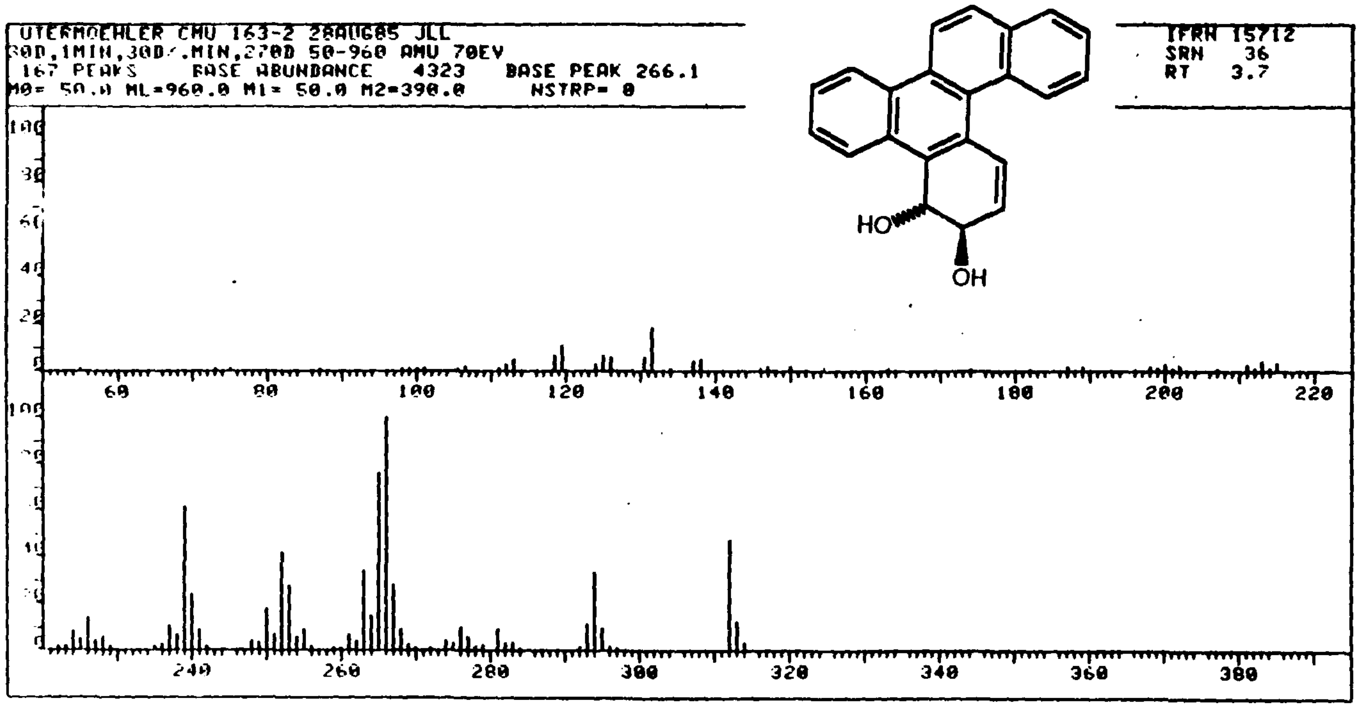
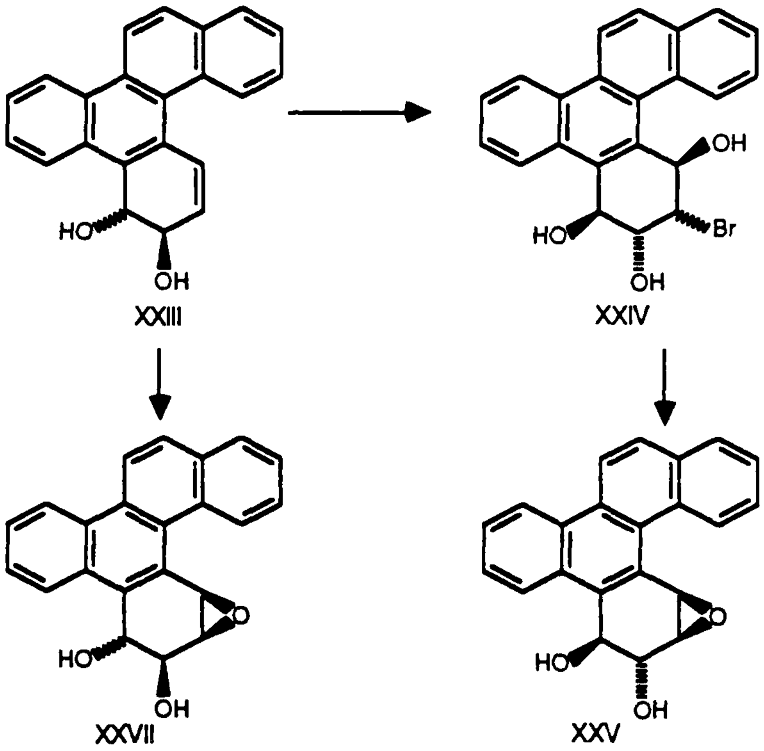


Figure 2.22 70 eV Mass Spectrum of XXIII

Scheme 2.12



However, an NMR spectrum of the crude material indicates that the benzo ring may have aromatized.

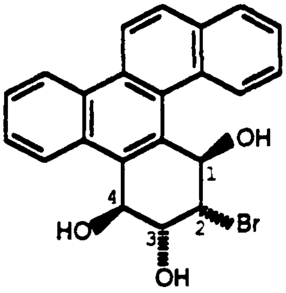
As an alternative, hydroxybromination conditions were slightly changed. Instead of excess NBA, only a 1:1 ratio was used. In addition, the amount of HCl was greatly changed - now one half drop of 6N HCl was added as the catalyst. After one hour at 0° the reaction was stopped. Purification was by column chromatography using dry column grade silica gel. Benzene was the first solvent, followed by 30% ethyl acetate/benzene, then 50% ethyl acetate/benzene. After evaporation of the solvent and trituration with petroleum ether, an off white solid melting at 87-90°C was produced. Yields in the synthesis of XXIV using this modified procedure were low (26%), yet provided adequate material for further conversion. NMR results: δ=8.99(m, 1H), 8.76(m, 1H), 8.66(d, J=9.22Hz, 1H), 8.42(m, 1H), 8.01(m, 2H), 7.36-7.75(m, 4H), 6.26(dd, J=5.65Hz, 5.43Hz, 1H), 5.69(dd, J=4.23Hz, 3.69Hz, 1H), 4.84(m, 2H), 3.30(d, J=5.65Hz, 1H), 2.61(d, J=6.40Hz, 1H), 2.57(d, J=4.23Hz, 1H). See Figures 2.23 through 2.25.

Assignment of protons and hydroxyl connectivity in this NMR spectrum (Figures 2.23-2.24) was by NMR decoupling experiments. The mass spectrum of XXIV did not provide a molecular ion, even at 12 ev. However, a typical mono brominated pattern is seen at m/e of 372 and 374. This is very likely due to the loss of two molecules of water, either by thermal dehydration or electron impact.

$$\underset{\text{m/e 408}}{[C_{22}H_{17}O_3Br]^{+\cdot}} \xrightarrow{-2H_2O} \underset{\text{m/e 372}}{[C_{22}H_{13}OBr]^{+\cdot}}$$

Figure 2.23

XXIV Proton NMR Assignments



XXIV

Proton	NMR Data (ppm)
1	6.26
2	4.84
3	4.84
4	5.69
CH$_1$	3.30
OH$_3$	2.61
OH$_4$	2.57

The structure in Figure 2.23 places bromine in the 2-α position. This assignment is based on the reaction of XXIV with Amberlite resin ($^-$OH form) to form only diol epoxide XXV. See Scheme 2.13. In this reaction, epoxides XXVI and XXVII are possible products, if the 1-OH is α, and the 2-Br is β, since bromine is displaced by backside attack. However, only a single diol epoxide, XXV, is observed, as expected for XXIV. (α and β are used to signify below or above the plane of the page.)

Reaction of bromotriol XXIV to form diol epoxide XXV was straightforward. Amberlite IRA-400(OH) ion exchange resin purchased from Aldrich Chemical Company was prepared for reaction by placing a quantity in a narrow tube, as commonly used for column chromatography. Freshly dried THF is then passed through the resin at an approximate rate of

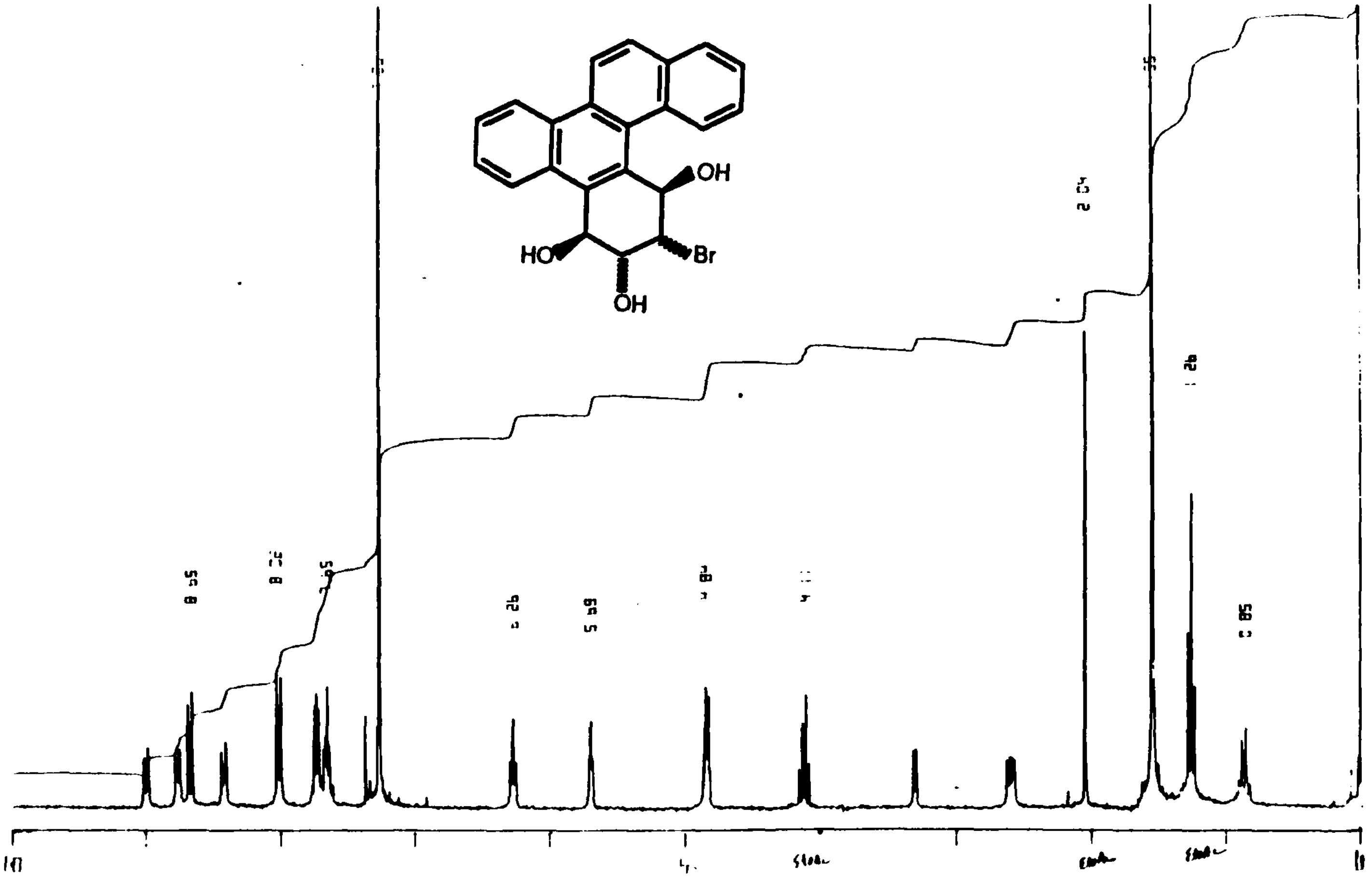


Figure 2.24 300 MHz NMR Spectrum ($CDCl_3$) of XXIV

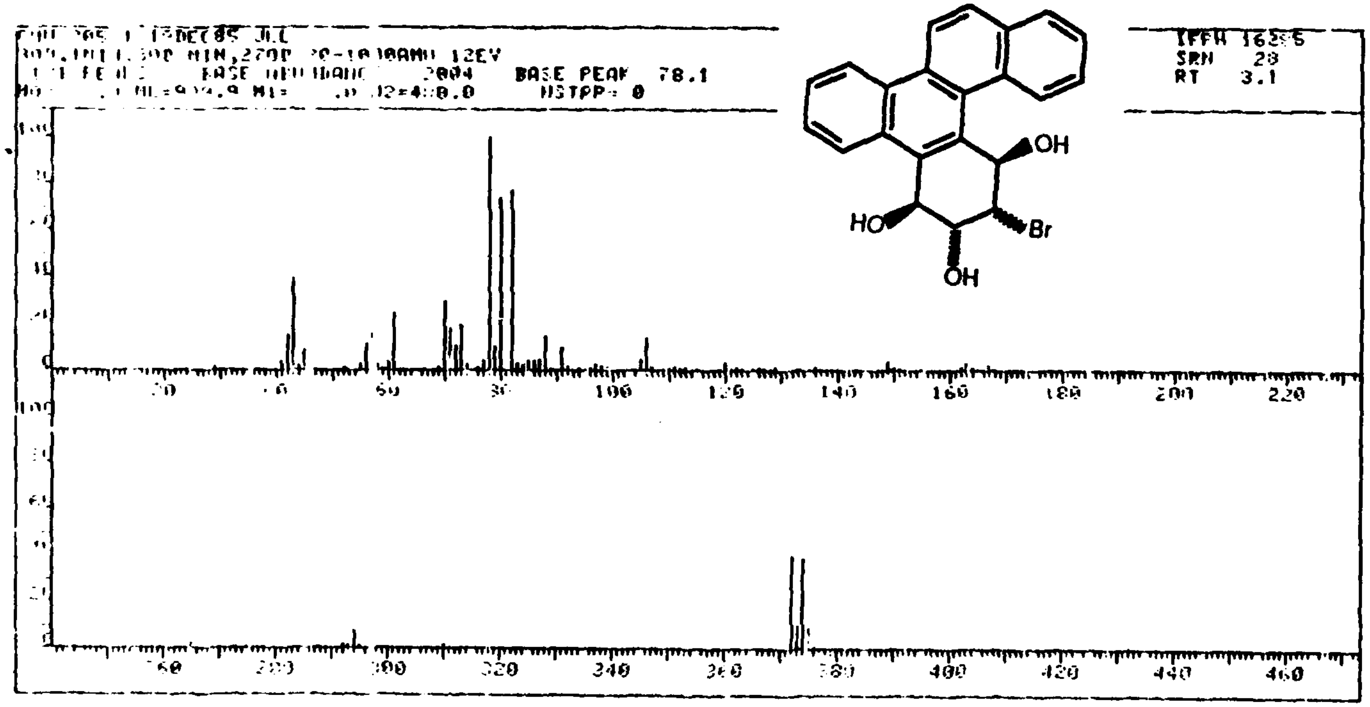
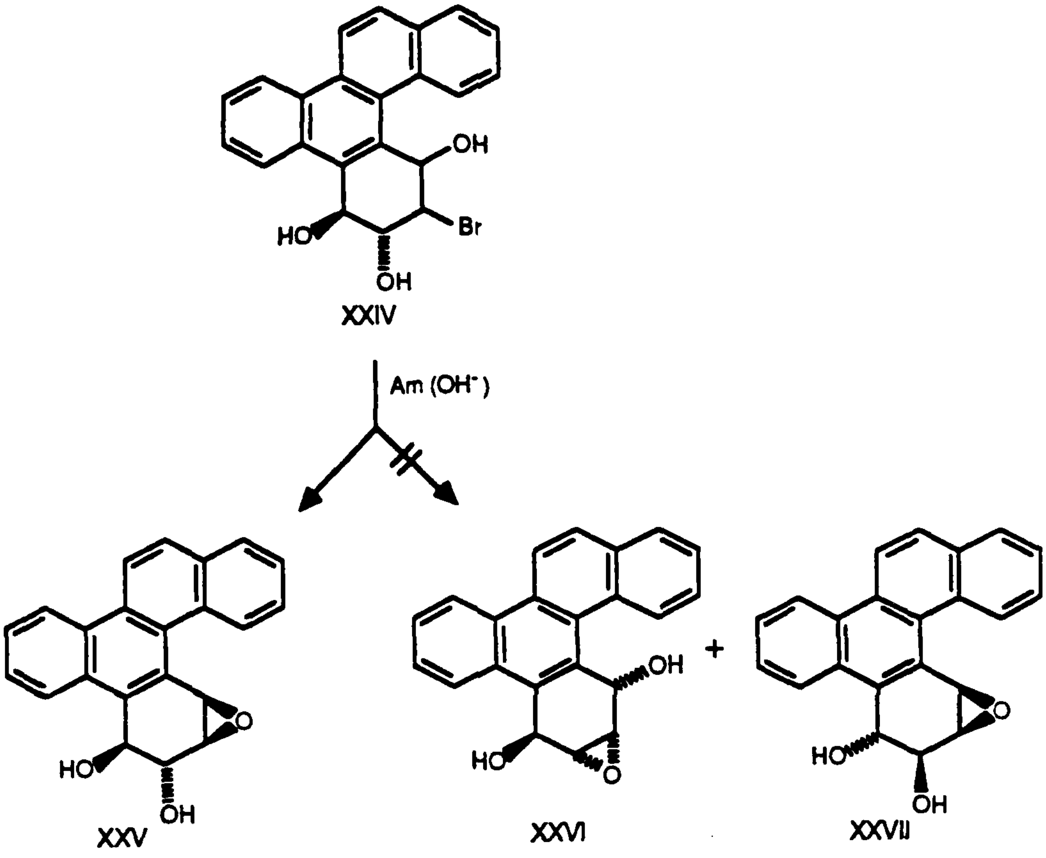


Figure 2.25 12 eV Mass Spectrum of XXIV

Scheme 2.13



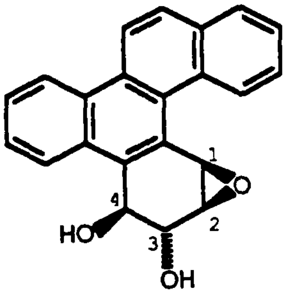
1-2 drops per second. When the resin turned dark brown in color it was ready for reaction. It was transferred to a flask and stored under freshly dried THF.

A large excess of resin in freshly dried THF was stirred with XXIV for 3 hours at room temperature and protected from light. The reaction mixture was quickly filtered and the THF was evaporated to give 78% yield of diol epoxide as a white solid, melting point 134-137°C.

NMR ($CDCl_3$) results for XXV: δ=8.76(m, 1H), 8.68(m, 2H), 8.38(m, 1H), 8.03(m, 2H), 7.64-7.78(m, 4H), 5.65(m, H_4), 5.15(d, J=4.0Hz, H_1), 4.91(m, H_3), 4.06(m, H_2), 2.87(d, J=11.9H_2, OH_4), 1.36(d, J=8.7Hz, OH_3). See Figures 2.26 thru 2.28. Figures 2.26 contains coupling constants determined through decoupling experiments.

Figure 2.26

Coupling Constants (Hz) for XXV



XXV

$J_{1,2}$ = 4.0
$J_{2,3}$ = 2.6
$J_{2,4}$ = 1.7 (w)
$J_{3,4}$ = 2.7
J_{3,OH_3} = 8.7
J_{4,OH_4} = 11.9

The high resolution mass spectrum of this sample gave a molecular ion of mass 328.1093±.0034, in excellent agreement with the calculated value for $C_{22}H_{16}O_3$ of 328.1099. The base peak in the 12ev spectrum is: m/e 328. Fragmentation in this spectrum occurs to give major m/e values at 310, 282, and 268.

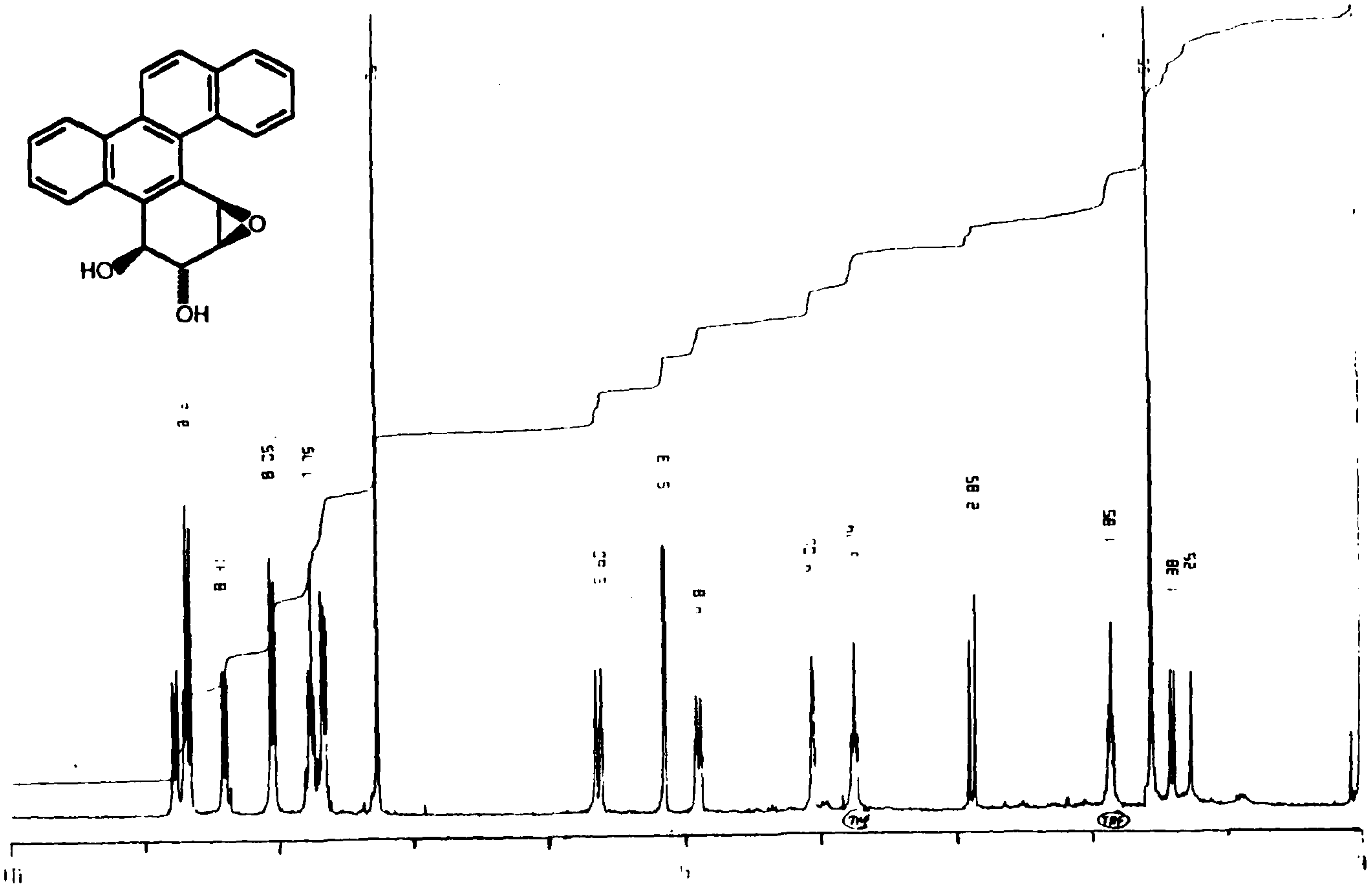


Figure 2.27 300 MHz NMR Spectrum ($CDCl_3$) of XXV

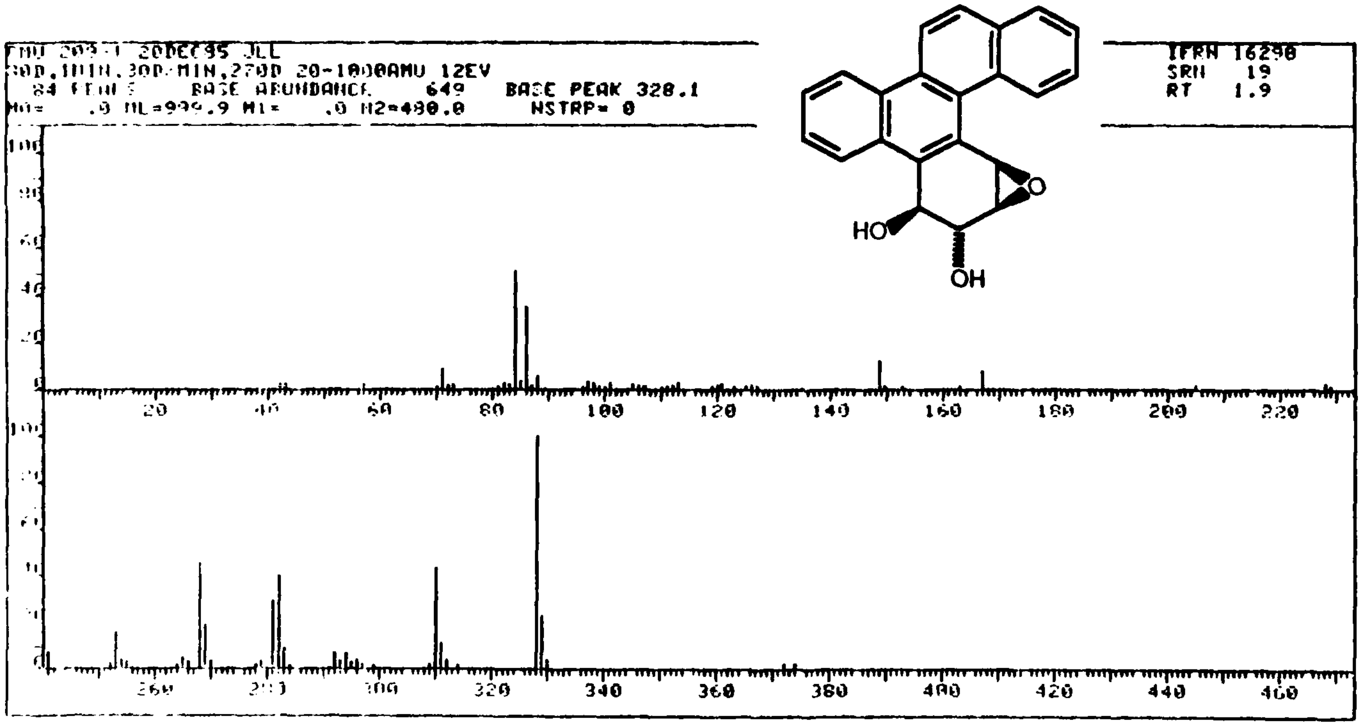
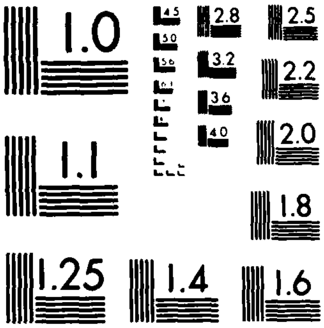


Figure 2.28 12 eV Mass Spectrum of XXV

AD-A171 766 SYNTHESIS OF POTENTIAL METABOLITERS IN THE 1234 AND 2/3
5678 BENZO RING POSIT..(U) AIR FORCE INST OF TECH
WRIGHT-PATTERSON AFB OH C M UTERMOEHLEN 1986
UNCLASSIFIED AFIT/CI/NR-86-139D F/G 7/3 NL



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Trace amounts at m/e 372 and 374 are due to the precursor bromotriol (Figure 2.28).

Since only the cis diol epoxide was prepared using the bromotriol route, prospects looked good for preparing the trans diol epoxide from peroxyacids. However, we weren't successful in obtaining exclusive formation of trans diol epoxide XXVII.

Initial attempts at preparing XXVII followed earlier procedures (23) with m-chloroperoxybenzoic acid (mCPBA). Dihydro diol (XXIII) was stirred at room temperature in the presence of excess mCPBA in THF for 40 minutes. Work up consisted of adding ether, and then washing the organic layer with cold 2% NaOH solution, water, drying (Na_2SO_4) and evaporating the solvent. This procedure provided a myriad of products that made it virtually impossible to isolate diol epoxide. A number of variations on the basic procedure were tried, in addition to other solvent systems. These variations are summarized in Table 2.13.

Table 2.13

Attempted Synthesis of XXVII

Solvent	Equiv mCPBA	Rxn time	Rxn temp	%Rxn	%trans/ %cis
THF	11	16hr	R.T.	100	-
THF	1.1	3hr	R.T.	50	70/30
THF	11	40min	R.T.	100	70/30
CH_2Cl_2	1.5	2hr	R.T.	100	0
CH_2Cl_2/5%$NaHCO_3$	1.5	2hr	R.T.	100	0

Table 2.13 (cont.)

Solvent	Equiv mCPBA	Rxn time	Rxn temp	%Rxn	%trans/ %cis
THF	1.1	4hr	0	30	70/30
THF	11	1.5hr	0	50	75/25
THF	11	3.5hr	0	71	71/29
THF	11	5hr	0	100	80/20
CH_3CN	5	2.5hr	0	70	69/31
THF	11	16hr	-20	<5	-
THF	11	4hr	-5	22	75/25
THF	11	4hr	15	100	86/14

The reaction wasn't as clean as the Table indicates. In addition to the two diol epoxides, additional absorptions in the aromatic region at δ=7.5ppm indicated another product was being produced. No attempts to isolate this compound were made; however, m-chlorobenzoic acid addition products seem likely. This product formed in all cases (by NMR); however, varying reaction temperature affected the amount of adduct formed. Cooler temperatures gave lower amounts of adduct and essentially gave the same ratio of trans to cis diol epoxide isomers. Lower temperatures also reduced other side reactions and provided an overall cleaner reaction.

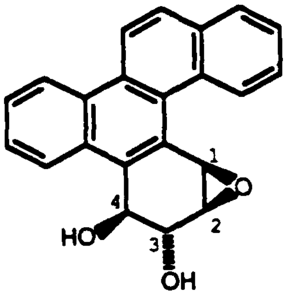
As a result, conditions selected for this reaction were a 10 fold excess of mCPBA in THF at 10-15°C. Reaction progress was best followed by ultraviolet absorption spectra. Dihydrodiol XXIII absorbed in the UV at 343nm while the diol epoxides do not. As such, the reaction was stirred at 10-15°C until the absorption at 343nm was absent (four hours).

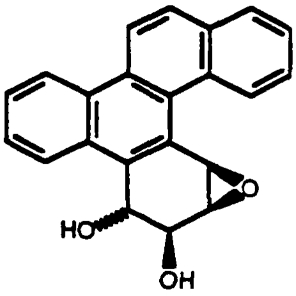
Purification of the reaction products proved to be quite difficult. We were unable to recover any diol epoxide using column chromatography with either silica or alumina columns. By using flash chromatography, the principal product collected was the cis diol epoxide, with only traces of the trans isomer. Although TLC experiments indicated that there was no separation of the two epoxide isomers, we attempted separation using high performance liquid chromatography (HPLC). Initial results were the same as that for flash chromatography, e.g., only minor amounts of diol epoxides were recoverable. However, HPLC easily removed the "benzoic acid derivative" from the product mixture. It appeared that HPLC had potential to at least separate the two diol epoxide isomers from other contaminants. Earlier workers (24) found they could separate diol epoxides using a THF/hexane solvent system. However, to recover substantial diol epoxides and avoid apparent decomposition while in the column, I found that it was necesary to cool the HPLC column. To do so the column was wrapped with tubing and a circulating pump was used to pass through ice cold water. Although the column temperature was lowered at most 10° from room temperature it was sufficient to permit recovery of diol epoxides in the same ratio as that injected, as judged by NMR.

Further attempts to separate the two diol epoxides were not successful. However, this material was prepared for mutagenicity studies and should prove useful to that end. Comparisons of reactivity will be possible since the <u>cis</u> diol epoxide was prepared as a single isomer in the bromo triol route. Changes in activity, if any, from the studies on the mixture versus studies on the <u>cis</u> isomer can be attributed to the <u>trans</u> isomer.

Table 2.14

NMR Data for Diol Epoxides XXV and XXVII

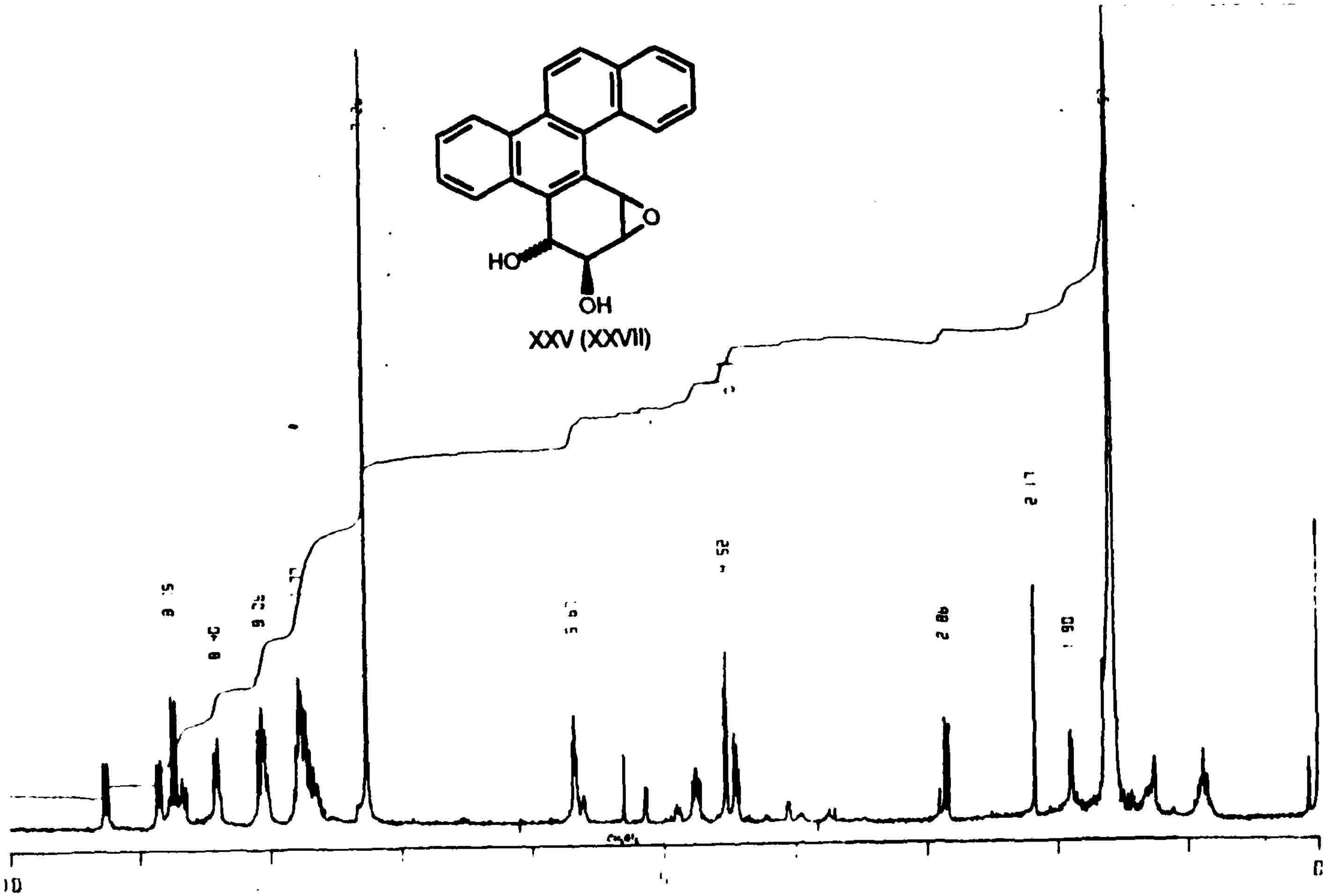




H	(ppm)	H	(ppm)
1	5.15	1	4.51
2	4.06	2	4.45
3	4.91	3	4.75
4	5.65	4	5.66
14	8.78	14	9.27

Coupling Data (Hz)

$J_{1,2}=4.0$	$J_{1,2}=4.0$
$J_{2,3}=2.6$	$J_{2,3}=6.0$
$J_{3,4}=2.7$	$J_{3,4}=3.1$
$J_{2,4}=1.7$	$J_{2,4}=0$



XXV (XXVII)

Figure 2.29 300 MHz NMR Spectrum ($CDCl_3$) of 86/14 mixture of XXVII/XXV

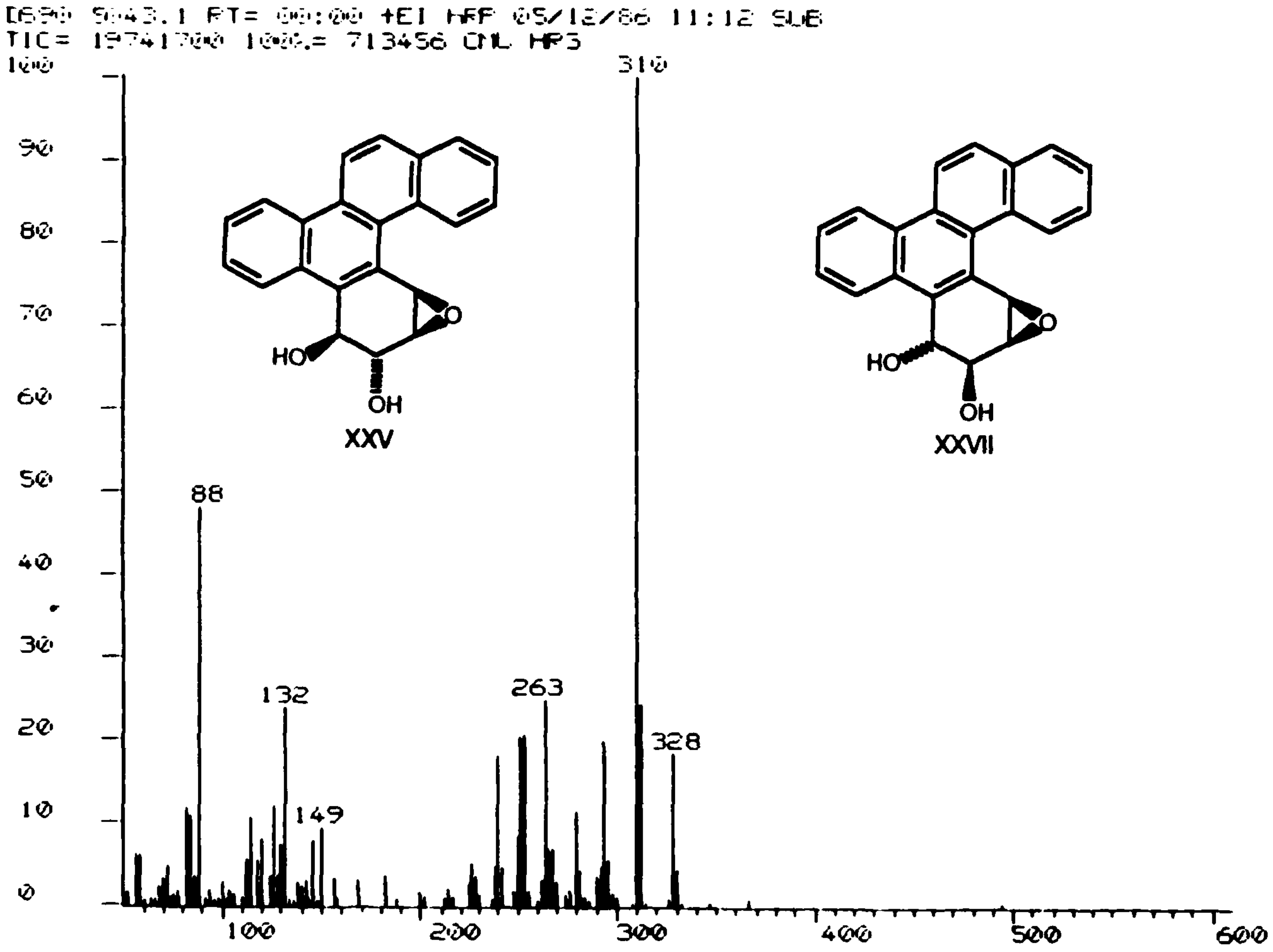


Figure 2.30 High Resolution Mass Spectrum of 86/14 mixture of XXVII/XXV

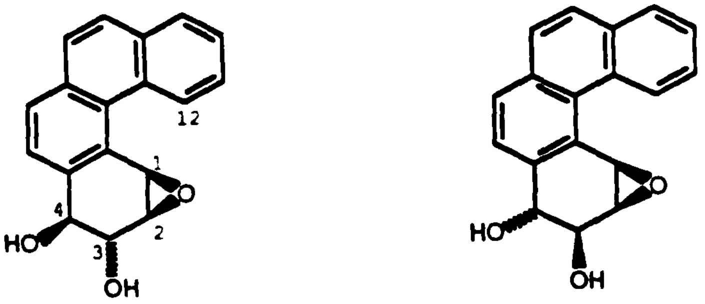
The high resolution mass spectrum for this mixture gave a molecular ion of 328.1083$\pm$.0023. Calculated value for $C_{22}H_{16}O_3$ is 328.1099. NMR data ($CDCl_3$) is shown in Table 2.14. (See also Figures 2.29 and 2.30.)

NMR data is shown in Table 2.14 for both diol epoxides made in this section. In previous work it was noted that unless there are unusual steric constraints placed on the hydroxyls, and the benzylic hydroxyl group is _cis_ to the epoxide oxygen, diol epoxides prefer the conformation where the hydroxyl groups are diaxial. When the benzylic hydroxyl group is _trans_ to the oxirane, the preferred conformation is quasi diequatorial.

These conformational arrangements have shown marked differences in tumorigenic activity. As is normally the case, bay region diol epoxides with quasi diequatorial hydroxyl groups show activity while those with quasi diaxial hydroxyls are weak to inactive. A model for comparison is the diol epoxides of benzo(c)phenanthrene (Figure 2.31).

Figure 2.31

Diol Epoxides of Benzo(c)phenanthrene



cis(series 1) *trans*(series 2)

These diol epoxides, in particular, series 1 diol epoxide are unusual because both isomers adopt a preferred quasi diequatorial conformation for the hydroxyl groups. This has been attributed to the steric hindrance that is present between protons at carbons 1 and 12. Both series 1 and 2 displayed high biological activity in terms of mutagenicity and tumorigenicity studies (25, 26).

Although the axial versus equatorial hydroxyl arrangement may not be the only factor in determining biological activity (27) it is apparent that it is a contributing factor in results reported thus far. Determination of this arrangement can be approximated by observation of models. However, ^{1}H NMR provides that information directly by analysis of the coupling constants for protons attached to carbons 3 and 4.

Table 2.15

NMR Data for Benzo(c)Phenanthrene Diol Epoxides(8)

series 1		series 2	
H	(ppm)	H	(ppm)
1	4.32	1	4.71
2	3.87	2	3.69
3	3.60	3	3.76
4	4.64	4	4.60
12	9.05	12	8.60

Coupling Data (Hz)

$J_{1,2}=4.0$	$J_{1,2}=4.0$
$J_{2,3}\sim 1.0$	$J_{2,3}=2.0$
$J_{3,4}=9.0$	$J_{3,4}=8.0$

For hydroxyls to be diequatorial, associated protons must be diaxial and the coupling constant should be large (greater then 7 by Karplus predictions)(7). As can be seen in Table 2.15, the coupling constants, $J_{3,4}$ for both epoxides exceed 7 Hertz and the hydroxyl groups are considered quasi diequatorial.

By similar reasoning, and by examining molecular models, if hydroxyl groups of diol epoxides XXV and XXVII prefer diequatorial conformations, the dihedral angle between protons 3 and 4 will approach 180°. In other words the coupling constant should be close to 7 or 8 Hertz. In the quasi diaxial conformation, the angles approach 60° or coupling between 1 and 5 Hertz. From Table 2.14, actual coupling constants were found to be 2.7 and 3.1 Hertz. Therefore both epoxides (series 1 and series 2) prefer quasi diaxial conformations for their hydroxyl groups. It appears that steric factors play a predominant role in maintaining these preferred conformations. In benzo(c)phenanthrene there was no "outside" steric interference with either hydroxyl group. Hindrance at the C_1 and C_{12} protons provided the impetus for the diequatorial conformation of the hydroxyls.

With diol epoxides XXV and XXVII there is additional substantial steric repulsion between the C_5-hydrogen and the benzylic hydroxyl group in both diequatorial conformations. The diaxial conformation relieves strain at C_4 and C_5 leading to the preferred arrangement. This arrangement is depicted in Figure 2.32.

Figure 2.32

Quasi Diaxial Conformations of XXV and XXVII

XXV

XXVII

There are two other interesting results available from NMR experiments. First, diol epoxide XXV (series 1), shows a $^4J_{2,4}$-W coupling of 1.7Hz. This indicates that the benzylic carbinol hydrogen bond and the nonbenzylic oxirane hydrogen bond are nearly coplanar and have a relatively fixed conformation. This coupling value corresponds quite well with W coupling found in the quasi diaxial arrangements of the series-1 benzo(e)pyrene 9,10-diol-11,12-epoxide (2.5Hz) and triphenylene 1,2-diol-3,4-epoxide (1.7Hz) (24).

Secondly, in diol epoxide XXVII (series 2), the benzylic oxirane hydrogen shows a 0.6ppm upfield shift relative to the same hydrogen atom on XXV. This is consistent with the proton at C_1 being tilted away from the aromatic plane and receiving less aromatic deshielding than the corresponding proton in XXV. In addition there is a substantial downfield shift (approximately 0.5ppm) in the proton at C_{14}. This can be attributed to the proximity of the C_{14}-hydrogen atom to the oxirane oxygen in XXVII as compared to XXV. This phenomenon is also observed in benzo(c)phenanthrene diol epoxides at C_{12}. However, in this case, the diequatorial conformations are preferred and series 1 diol epoxide demonstrates a 0.45ppm downfield shift over series 2.

Early reports concerning the "bay-region" theory predicted that benzo ring diol epoxides in which the epoxide occupied the bay region should be the most biologically active. In addition to that criterion, recent reports now include conformation information related to the vicinal hydroxyls. One could predict that a bay region epoxide of carcinogenic PAHs with quasi diequatorial hydroxyls is or should be a highly active carcinogen. A corollary to this prediction implies if the bay region epoxide contains diaxial hydroxyls, the diol epoxide is mildly active to biologically inactive.

Based on these guidelines one would almost certainly predict low activity for both diol epoxides XXV and XXVII. This is based solely on results of benzo(e)pyrene and triphenylene in which the hydroxyls are quasi diaxial and for which the diol epoxides have lower mutagenicity and tumorigenicity than would be predicted by the calculated resonance energies. However, neither of these possesses a fjord region epoxide as in XXV, XXVII, and benzo(c)phenanthrene. The effect of this fjord region epoxide may be to develop another correlation scale relative to its own grouping of steric and conformational factors.

With fairly good certainty, one could predict that the quasidiaxial arrangement of the hydroxyls will cause reduction in biological activity when compared with the results of benzo(c)phenanthrene with its quasi diequatorial conformation. However, it would be currently incorrect to predict relative reactivity of XXV or XXVII using data solely from "bay region" diol epoxides.

SUMMARY

In this chapter, the preparation of racemic 3,4-dihydroxy-1,2-epoxy-1,2,3,4-tetrahydrobenzo(g)chrysene, isomer 1 (XXV) and isomer 2 (XXVII) and their structural characterization were described. Photochemical methods were applied in the direct synthesis of a 5-position substituted chrysene that exceeded a two carbon substituent. In addition, potential metabolic intermediates (XIX, XXIII) leading to XXV and XXVII were synthesized.

Tumorigenicity and mutagenicity studies haven't yet been conducted on these diol epoxides. However, a discussion of how these fjord region epoxides may correspond to previously studied "bay region" epoxides is included. One could predict that biological activity for XXV and XXVII will be lower than for the corresponding fjord region diol epoxides of benzo(c)phenanthrene due to the quasidiaxial hydroxyl groups; however, the biological activity of fjord region diol epoxides with quasidiaxial hydroxyl groups has not yet been determined. It is unclear whether the effect for bay region diol epoxides with quasidiaxial hydroxyl groups will extend to the fjord region compounds.

EXPERIMENTAL

All melting points were obtained on a Kofler melting point apparatus and are uncorrected. Mass spectra were obtained on a Hewlett-Packard 5985 quadrupole mass spectrometer operated at 12 or 70ev. High resolution mass spectra were obtained using a Kratos MS25RF mass spectrometer using perfluorokerosene as the internal reference. 300 MHz PMR spectra were obtained on a Varian XL-300 spectrometer. Ultraviolet spectra were obtained using a Perkin-Elmer Lambda 3 spectrophotometer. High Performance Liquid Chromatography was accomplished on a Beckman model 330 Liquid Chromatograph with Altex Ultrasphere-Si 10mm x 25cm columns. Preparative photolysis was accomplished using a Rayonet RS Preparative photochemical reactor at 300nm. Cyclohexane was prepared for photolysis reactions by treating with concentrated sulfuric acid before distillation. Hexane was also treated with concentrated sulfuric acid, washed and distilled from calcium sulfate. Super dry ethanol refers to absolute ethanol that was refluxed with magnesium metal and iodine for two hours then distilled prior to use. Tetrahydrofuran was dried by reflux and distillation from calcium hydride.

Anhydrous benzene was prepared by reflux over sodium metal followed by distillation. Microanalyses were performed by Galbraith Laboratories, Inc., Knoxville, Tennessee.

1-(1-naphthyl)-cyclopentan-1-ol (X)

5.670g of magnesium (0.233mole) was placed in a three necked flask equipped with mechanical stirrer, reflux condenser with drying tube, and dropping funnel. This apparatus was flame dried and allowed to cool by passing a stream of nitrogen through the apparatus. 25ml of anhydrous ethyl ether, 2g of 1-bromonaphthalene, and a crystal of I_2 were added and the mixture was brought to a boil by heating with a water bath. When the Grignard reagent began forming, stirring was started and the remaining 48g of 1-bromonaphthalene (50g total, .242mole), was added in 75ml of anhydrous ether over 2 hours. Reflux was continued until formation of the Grignard reagent was complete. Before cooling, 75 ml of anhydrous benzene was added to dissolve the Grignard reagent. The mixture was cooled via ice bath and 19.8g of cyclopentanone (.236 mole) was added dropwise. The mixture was then stirred at room temperature for 1-2 hours, followed by dropwise addition of 20% NH_4Cl solution. This mixture was then washed twice with 100ml water, once with saturated sodium chloride, and the solvent was evaporated using a rotovapor at reduced pressure. Purification was accomplished using flash chromatography with benzene as the solvent. After removal of solvent by rotovapor, the procedure gave 24.02g of X (49%) as a white solid, with melting range 74.5-75.5°C (lit. 75.5-76°)(12). NMR: 8.66(d, 7.7Hz, 1H), 7.87(d, 7.1Hz, 1H), 7.78(d, 8.1Hz, 1H), 7.60(d, 7.3Hz, 1H), 7.30-7.59(m, 3H), 1.84-2.35(m, 9H).

1-(1-naphthyl)-1-cyclopentene (XI)

10.10g of alcohol X was dissolved in 100ml of benzene in a 250ml round bottomed flask equipped with magnetic stirrer and reflux condenser. 14.5mg of *para*-toluene sulfonic acid was added and the reaction mixture was refluxed. The reaction progress was easily monitored using thin layer chromatography with 1% ethyl acetate in benzene as the solvent. After two hours of reflux, the mixture was cooled to room temperature and transferred to a 250ml separatory funnel. The organic layer was washed 2x with 100ml water, dried (Na_2SO_4) and the solvent was removed under reduced pressure to give a quantitative yield of XI.

Alternatively, better overall conversion to XI was achieved by not isolating alcohol X. Instead, after hydrolysis and washings, the organic layer is concentrated and benzene is added to bring the volume to 100ml. Dehydration is accomplished as already described. After cooling and washing with water, the solvent was removed by evaporation and the residue was distilled via Kugelrohr at 1-2 torr. As the Kugelrohr oven heated, the early fractions were discarded. The oven range was 150-170°C during collection to give a 74% yield of XI as a colorless oil. NMR: 8.29(m, 1H), 7.9(m, 2H), 7.4-7.6(m, 4H), 6.06(m, 1H), 2.93(m, 2H), 2.75(m, 2H), 2.21(m, 2H).

4-(1-naphthoyl) butyric acid (XII)

Method 1. 2.00g of carbinol X was dissolved in 70ml of glacial acetic acid with magnetic stirring. To the mixture was slowly added 12g of chromium trioxide while maintaining the temperature below 30°C with an ice bath. After two hours of stirring at room temperature the mixture was diluted with 70ml of water and extracted four times with 300ml of ethyl ether. The organic layer was then washed 4x with 100ml of water and then extracted with 100ml of 15% sodium hydroxide solution. The base extracts were combined, acidified with concentrated hydrochloric acid, and extracted with ether to give 0.723g of crude XII (32%).

Method 2. 12.0g of alkene XI and 1.0g of 18-crown-6 ether were dissolved in 1.0 liter of tetrahydrofuran (THF) in a 2 liter round bottomed flask equipped with magnetic stirrer. Potassium permanganate (20.52g) was added and the reaction was stirred for 3 hours at room temperature. After 3 hours a TLC (silica gel, benzene) indicated alkene was still present; however, increasing the reaction time also caused further oxidation to naphthoic acid. Sodium bisulfite and 6N hydrochloric acid were added alternately up to 14g of $NaHSO_3$ and 200ml 6N HCl. At this time all the formed manganese dioxide dissolved and the mixture was colorless. THF was removed under reduced pressure and the residue was extracted with methylene chloride. The organic layer was then extracted with 150ml portions of 0.5N sodium hydroxide. The aqueous layer was then acidified (concentrated HCl) and extracted with methylene chloride. After washing with saturated salt solution and drying (Na_2SO_4), the methylene chloride was removed under vacuum. The procedure was repeated with 11.3g of alkene XI and the combined product was 7.154g crude keto acid XII. 15.16g of XI was recovered from the neutral residue and redistilled by Kugelrohr. Correcting for recovered starting material gave a crude yield of 70.5%.

4-(1-naphthoyl) methyl butyrate (XIII)

Method 1. Keto acid (XII) prepared from either method above was refluxed in anhydrous methanol (100ml) containing 1ml of concentrated hydrochloric acid. After three hours of reflux, the methanol was removed under reduced pressure. The residue was dissolved in methylene chloride, washed with water, dilute base, dried (Na_2SO_4) and the solvent was removed with rotovapor. Purification was by flash chromatography using benzene as the solvent. Evaporation of solvent provided XIII. Yield of XIII by method I was 32%. Yield of XIII by method 2 was 45%.

Method 2. This method generally followed the procedure of Wiberg and O'Donnell (28). Naphthalene (15.61g, 0.122 mole) was placed in a 3 necked flask equipped with reflux condenser, overhead stirrer, and dropping funnel. Methylene chloride (100ml) was added and cooled to $0^{\circ}C$ via ice bath. Aluminum chloride (32.68g, 0.246mole) was added and the reaction mixture was stirred for 10 minutes. Methyl 4-(chloroformyl)butyrate (20.0g, .122 mole) in 25ml of methylene chloride was added dropwise while cooling was continued. The reaction was allowed to stir overnight at room temperature. The next day water was added dropwise, with cooling. When the aluminum salts were dissolved, the mixture was transferred to a 250ml separatory funnel and the organic layer was separated and washed with 100ml each of water, .5N sodium hydroxide, dilute hydrochloric acid, water and saturated salt solution. Anhydrous sodium sulfate was added, the dried organic solution was decanted and the solvent was evaporated via rotovapor.

The residue was purified by distillation via Kugelrohr at 0.5 torr. A first fraction distilled at 150-180°C and was discarded as the dimethyl ester of glutaric acid. The second fraction was collected at 210-220°C. (18.43g). 100ml of absolute ethanol was added to this residue and petroleum ether was added dropwise until the solution became slightly cloudy. This was then placed in a -20°C freezer overnight. By the next morning a solid crystallized out and was filtered off. This solid proved to be the 2-naphthoyl derivative of XIII (see Chapter V). Ethanol was removed at reduced pressure leaving 14.34g of XIII (45.9% yield) as a light yellow oil. NMR data: 8.58(d, J=3.8Hz, 1H), 7.98(d, J=8.3Hz, 1H), 7.87(d, J=7.3Hz, 2H), 7.4-7.6(m, 3H), 3.68(s, 3H), 3.13(t, J=7.3hz, 2H), 2.48(t, J=7.1Hz, 2H), 2.14(p, J=7.3, 7.1Hz, 2H). Mass spectrum m/e 256, 238, 172, 155.

<u>5-(1-naphthyl)-6-phenyl-ethyl-hex-5-enate (XIV)</u>

Sodium ethoxide was freshly prepared by adding 1.675g (.0728mole) sodium metal to 88ml of "super dry" ethanol. This was added dropwise via dropping funnel to 28.331g (.0728mole) benzyl triphenyl phosphonium chloride dissolved in 80ml "super dry" ethanol in a 250ml round bottomed flask equipped with magnetic stirrer. Stirring was continued for an hour after addition was completed. At that time 14.34g (.0560mole) of keto ester XIII in 50ml ethanol was added to the flask and the dropping funnel was replaced with a reflux condenser capped with a drying tube. The mixture was then refluxed for 72 hours. Ethanol was removed by evaporation at reduced pressure and the residue was dissolved in methylene chloride, washed with water, dried (Na_2SO_4) and solvent was removed by rotovapor. Benzene was added to dissolve the residue and petroleum ether was added dropwise to precipitate the triphenylphosphine oxide. The precipitate was filtered off and washed with additional benzene/petroleum ether solution. The organic solution was concentrated. Residual traces of phosphine oxide could then be removed with final purification using flash chromatography on silica gel. The initial solvent was petroleum ether followed by benzene to elute the stilbene XIV. Most of the solvent was removed by rotovapor, with final traces being removed using the Kugelrohr (1mm, 100°C.) to yield 16.558g XIV (86%). No attempt to separate isomers was made. NMR showed 67% *Z* isomer (δ=6.62) and 33% *E* isomer (δ=6.75). Mass spectrum m/e 344, 229, 165.

4-(5-chrysenyl)-ethyl butyrate (XV)

A Pyrex photolysis flask was equipped with a gas inlet bubbler, a reflux condenser, and magnetic stir bar. To this flask was added 1.55g (.00472mole) of stilbene XIV, 1.5 liter of freshly distilled cyclohexane, and 80mg iodine. The solution was saturated with dry oxygen by passing pressurized air through a potassium hydroxide/calcium sulfate drying apparatus enroute to the bubbler. Stirring was initiated and photolysis with 300nm wavelength light began. The reaction was more than 90% complete after 3 days as judged by NMR and the photolysis was halted. Cyclohexane was removed by rotovapor and the residue was redissolved in methylene chloride, concentrated and crudely purified by flash chromatography on a 5.5cm column of silica gel. 300ml of petroleum ether was the first solvent and removed residual iodine. This was followed by 10% ether in petroleum ether to elude 1.10g crude product, 77% by NMR was XV which was characterized by a 3H downfield multiplet in the NMR spectrum at δ=8.70-8.81. Best results were obtained by repetition of 1.5 to 1.7g reactions as compared to larger scale.

4-(5-chrysenyl)butyric acid (XVI)

Butyric ester XV was hydrolyzed by aqueous potassium hydroxide. 6.374g of XV was transferred, using 10ml of tetrahydrofuran, to a 250ml round bottomed flask equipped with reflux condenser, magnetic stirrer, and heating mantle. To this flask was then added a solution of 10g potassium hydroxide dissolved in 180ml of water. Reflux was initiated and continued for 3.5 hours. After cooling to room temperature the solution was transferred to a separatory funnel and washed 2x with 100ml ether. The aqueous layer was then acidified with hydrochloric acid forming a white precipitate. The acidified material was extracted using methylene chloride, dried (Na_2SO_4), and the solvent was removed under reduced pressure. The oil produced was crystallized using ethyl ether and the solid was collected by filtration. The crystals were washed with cold ether to give 3.4531g of XVI, melting range of 161-165°C. NMR data: 8.6-8.8(m, 3H), 7.8-8.0(m, 4H), 7.4-7.7(m, 4H), 3.65(bt, 2H), 2.52(t, 7.2Hz, 2H), 2.29(m, 2H). Mass spectrum m/e 314, 254, 241.

1,2-dihydrobenzo(g)chrysene-4(3H)-one (XVII)

To 3.4531g (.0110mole) of butyric acid XVI in a 100ml round bottomed flask was added 30ml of methanesulfonic acid. A magnetic stir bar was added and the flask was capped with a drying tube. Once acid XVI was covered with methanesulfonic acid, the flask was placed in an oil bath maintained at 55±2°C. After 2.5 hours, the darkened mixture was cooled to room temperature, poured slowly over crushed ice and stirred by glass rod. This aqueous mixture was extracted three times with methylene chloride (200ml total). The organic layer thus collected was washed with 100ml water, dried with sodium sulfate, and the solvent was removed under reduced pressure.

After sitting overnight in a -20°C freezer, the dark brown oil that was formed solidified. This was dissolved, with heating, in benzene and filtered through a short column of silica gel. Additional benzene (250ml) was used to ensure ketone removal from the column. Evaporation of the solvent gave 2.760g (.00932mole) of ketone XVII, 84.3% yield. If a deep yellow color remains at this point it may be removed by washing the solid with cold ethyl ether to give a cream colored solid, melting point 159-161.5°C. NMR data: 9.22-9.26(m, 1H), 8.52-8.74(m, 3H), 7.98-8.05(m, 2H), 7.61-7.72(m, 4H), 3.72(t, J=5.6Hz, 2H), 2.88(t, J=6.7Hz, 2H), 1.96(m, 2H). Mass spectrum m/e 296, 268, 239.

4-hydroxy-1,2,3,4-tetrahydrobenzo(g)chrysene (XVIII)

Ketone XVII (4.322g, 0.0146 mole) was placed in a 250ml round bottomed flask equipped with magnetic stirrer and external cooling (ice bath). Tetrahydrofuran (40ml) and 150ml of methanol were added, forming a suspension. (Additional tetrahydrofuran will increase the solubility, but wasn't necessary for the reaction of occur). Sodium borohydride (2.808g, 0.0739 mole) was added piecemeal over 15-20 minutes. (Rapid addition caused foaming and loss of material.) After the addition was complete, the ice bath was removed and the reaction mixture was stirred for one additional hour while capped with a balloon. This produced a white precipitate that was filtered. The precipitate was dissolved in 800-1000ml of methylene chloride, washed with water, dried, and the solvent was removed to give 3.585g of crude alcohol XVIII, melting point 195-8°C. NMR clearly indicated the carbinol proton as a broad singlet at δ=5.77. Mass spectrum m/e 298, 280, 241, 239, 119.

1,2-dihydrobenzo(g)chrysene (XIX)

The crude carbinol XVIII was suspended in 250ml of benzene in a 500ml round bottomed flask equipped with magnetic stirrer. To this suspension was added a few crystals of para-toluene sulfonic acid. The round bottomed flask was capped with a drying tube and the flask was covered with aluminum foil to prevent light-induced decomposition. After 16 hours of stirring at room temperature, the dehydration was complete and the solution was transferred to a 500ml separatory funnel and washed with water (2x, 100ml), dried with sodium sulfate, and concentrated under vacuum. The residue was placed on a dry column for flash chromatography and petroleum ether was used as the eluent giving 2.97g (0.0106mole, 68% from crude alcohol) XIX. The filtrate from ketone XVII reduction was also treated with para-toluene sulfonic acid in benzene and gave an additional 0.5276g of XIX (3.4932g total, 85.4% from ketone), melting point 140.5-143°C. NMR data: 7.5-8.77(m, 11H), 6.45(m, 1H), 3.65(t, J=8.1Hz, 2H), 2.25(m, 2H). High resolution mass spectrum m/e 280.1266±.0020. Calculated exact mass for $C_{22}H_{16}$ is 280.1253. UV(methanol): λ=211(181,000), 241(215,000), 268(228,000), 275(229,000), 338(86,500), 352(83,000).

trans-3,4-diacetoxy-1,2,3,4-tetrahydrobenzo(g)chrysene(XX)

In a 250ml round bottomed flask equipped with magnetic stirrer and heating mantle was placed 1.55g (0.00929mole) of silver acetate and 75ml of dried benzene. The mixture was stirred, and 1.179g (0.00464mole) of iodine was added. The flask was covered with aluminum foil, capped with a drying tube and stirred at room temperature 20-30 minutes. At that time, the red iodine color was gone leaving a yellow suspension. Alkene XIX (1.000g, 0.00357mole) was added and the mixture was stirred for 1 hour at room temperature. Progress of the reaction was easily followed using thin layer chromatography (TLC) with benzene solvent. A lower, darkened spot indicates the presence of the iodo acetate.

When TLC indicated the absence of XIX, a reflux condenser was added to the apparatus and reflux begun. After three hours, the reaction appeared complete, judged by loss of the iodo acetate spot in TLC and formation of yet another lower spot. The reaction mixture was filtered while hot and the filtrate was concentrated and placed on a flash chromatography column. Benzene was used as an initial solvent to remove traces of non-polar material, followed by 5% ethyl acetate to remove XX. Evaporation of the solvent by rotovapor and drying at 0.5 mm pressure gave XX as an aerosol (1.2852g, 90% yield). Crystallization using chloroform and petroleum ether gave a melting point of 128.5-131°C. NMR data: 8.6-8.77(m, 3H), 7.9-8.0(m, 3H), 7.6-7.7(m, 4H), 6.84(d, 3.1Hz, 1H), 5.48(m, 1H), 3.6-3.9(m, 2H), 2.19(s, 3H), 2.0-2.17(m, 5H). Mass spectrum m/e 398, 338, 326, 296, 278, 252, 239.

1-Bromo-3α,4β-diacetoxy-1,2,3,4-tetrahydrobenzo(g)chrysene (XXI)

Prior to beginning this reaction all glassware, separatory funnels, filter funnels and stir bars must be rinsed with dilute aqueous base such as ammonium hydroxide, rinsed with water, and thoroughly dried. Into a 250ml round bottomed flask equipped with magnetic stirrer and reflux condenser capped with drying tube was placed 600mg of XX (2.013mmole), 448mg of N-bromosuccinimide (2.732mmole), 4.6g sodium bicarbonate, and 180ml carbon tetrachloride.

The solution was saturated with nitrogen by bubbling gas for 10 minutes. The flask was placed in an oil bath and heated at 50-55°C and the solution was stirred for 2.5 hours. An aliquot was taken for NMR analysis and indicated that the reaction was complete. The solution was cooled to room temperature and transferred to a separatory funnel, washed twice with 100ml water, dried, and the solvent was removed by rotovapor without heating. The sample was kept an additional hour under 0.5mm pressure and XXI was used without further purification.

trans-3,4-diacetoxy-3,4-dihydrobenzo(g)chrysene (XXII)

Method 1. This method has the advantage in that the conversion from XX to XXII can be accomplished without intermediate work up of XXI. However, the disadvantage is the length of reaction time. In a typical experiment 250mg of XX (.628mole), 145mg N-bromosuccinimide and 2.0g sodium bicarbonate in 80ml carbon tetrachloride was reacted as described in XXI above. However, instead of stopping at 2.5 hours, the reaction continued on at 50-60°C for 120-180 hours. At that time the reaction material was poured into a separatory funnel and extracted twice with 100ml water, dried, and solvent was evaporated. The residue was purified by flash chromatography with 20% ethyl acetate in petroleum ether as the eluent. After evaporation of solvent, there remained 135mg of dihydro diacetoxy XXII (54.3% yield).

Method 2. The entire crude product XXI was dissolved in 40ml anhydrous THF and cooled to 0°. 1,4-Diazabicyclo[4.3.0] non-5-ene (DBN, 150 drops) was added. The mixture was stored under argon and placed in a freezer at -20°C for 16 hours. The reaction mixture was then poured into a separatory funnel, 50ml of ethyl acetate was added, and the organic layer was washed with 50ml each of water, 0.1N hydrochloric acid, 5% sodium bicarbonate solution, water, and saturated salt solution. The organic layer was dried (Na_2SO_4) and solvent was evaporated. After purification via flash chromatography (10% ethyl acetate in ether), evaporation of solvent, and trituration with ethyl ether, 138mg (23%) XXII was obtained.

Melting point: 154-156°C. NMR data: 8.48-8.80(m, 3H), 8.01-8.13(m, 3H), 7.63-7.73(m, 5H), 6.90(bs, 1H), 6.47(q, J=8.2, 5.9Hz, 1H), 5.49(dd, J=5.9, 1.7Hz, 1H), 2.08(s, 3H), 2.03(s, 3H). Mass spectrum m/e 396, 336, 294.

trans-3,4-dihydroxy-3,4-dihydrobenzo(g)chrysene(XXIII)

In a 250ml round bottomed flask equipped with a magnetic stirrer was placed 253mg (.639mmole) of XXII and 100ml of methanol. The solution was cooled to 0°C by ice bath and, while stirring, anhydrous ammonia was bubbled into the solution. After 15-20 minutes of bubbling, the ammonia saturated solution was capped with a balloon and the ice bath was removed. Stirring was continued at room temperature. After 16 hours, the methanol was removed under reduced pressure at room temperature and the residue was dissolved in methylene chloride. The organic layer was quickly washed with an equal volume of water, dried (Na_2SO_4), and the solvent was removed by rotovapor.

The product was purified by flash chromatography. However, the 10g of silica gel was first packed with benzene and kept from going dry. The product mixture was loaded using methylene chloride and initially eluted with 5% ethyl acetate in benzene to remove any aromatic phenols and less polar material. The solvent was changed to 50% ethyl acetate in petroleum ether and 400ml eluted XXIII as one spot on TLC (50% ethyl acetate/petroleum ether). The yield was 163mg (82%), melting point 149-151°C. NMR data: 8.45-8.79(m, 4H). 8.03(m, 2H), 7.58-7.75(m, 3H), 7.52(d, J=9.7Hz, 1H), 6.51(dd, J=9.7, 5.9Hz, 1H), 5.60(dd, J=7.3, 1.8Hz, 1H), 4.69(m, 1H), 1.89(d, J=7.3Hz, 1H(OH)), 1.70(d, J=7.3Hz, 1H(OH)). Mass spectrum m/e 312, 294, 267, 266, 265, 252. Analyzed for C,H. Calculated for $C_{22}H_{16}O_2$:C, 84.59; H, 5.16. Found: C, 84.50; H, 5.49.

(±)-2α-Bromo-1β,3α,4β-trihydroxy-1,2,3,4-tetrahydrobenzo(g)-chrysene (XXIV)

In a 25ml round bottomed flask containing a magnetic stir bar was placed dihydro diol XXIII (31.4mg, 0.101mmole), 6ml of freshly distilled tetrahydrofuran and 2ml of water. N-bromoacetamide (NBA, 13.9mg, .101mmole) was added, the reaction mixture was cooled to 0°C and stirring was begun. One drop of 6N hydrochloric acid was added and the reaction mixture was stirred for one hour. The flask contents were poured into 10ml of ethyl acetate and washed with 8ml each of water (2x) and saturated salt solution.

After drying (Na_2SO_4) and removing the solvent, a dry column was prepared and the material was eluted using 15ml benzene, then 15ml 30% ethyl acetate in benzene, followed by 50% ethyl acetate in benzene. After combining fractions and evaporating the solvent, and trituration with ether, 10.5mg (26%) of bromo triol XXIV was collected as an off-white solid. Melting point 87-90°C. NMR data: 8.99(m, 1H), 8.76(m, 1H), 8.66(d, J=9.22Hz, 1H), 8.42(m, 1H), 8.01(m, 2H), 7.35-7.75(m, 4H), 6.26(dd, J=5.65, 5.43Hz, 1H), 5.69(dd, J=4.23, 3.69Hz, 1H), 4.84(m, 2H), 3.30(d, J=5.65Hz, 1H), 2.61(d, J=6.40Hz, 1H), 2.57(d, J=4.23Hz, 1H). Mass spectrum (12eV) m/e 374, 372 (no molecular ion).

(±)-3α,4β-dihydroxy-1β,2β-epoxy-1,2,3,4-tetrahydrobenzo(g)-chrysene (isomer 1) (XXV).

In a 25ml round bottomed flask containing a magnetic stir bar was placed .75g of Amberlite IRA-(400)(OH) ion-exchange resin that had been freshly dried using anhydrous tetrahydrofuran (THF). Anhydrous THF was added or removed such that the resin was just covered. Bromotriol XXIV (10.0mg) was dissolved in anhydrous THF and added to the reaction flask. The flask was capped with a ground glass stopper and stirring was begun. After 3 hours at room temperature, the resin was removed by filtration using a cintered glass funnel and the THF was removed under reduced pressure to give 6.3mg (79%) of epoxide XXV. Melting point 134-137°C. NMR data: 8.64-8.77(m, 3H), 8.37-8.41(m, 1H), 8.05(m, 2H), 7.64-7.77(m, 4H), 5.65(m, $J_{2,4}$=1.7Hz, $J_{3,4}$=2.7Hz, $J_{4,OH}$=11.9Hz, H_4), 5.15(d, $J_{1,2}$=4.0Hz, H_1), 4.91(m, $J_{3,4}$=2.7Hz, $J_{2,3}$=2.6Hz, $J_{3,OH}$=8.7Hz, H_3), 4.06(m, $J_{2,4}$=1.7Hz, $J_{1,2}$=4.0Hz, $J_{2,3}$=2.6Hz, H_2), 2.85(d, J=11.9Hz), 1.38(d, J=8.7Hz). Mass spectrum m/e 328, 310, 282, 268, 253. High resolution mass spectrum m/e 328.1093±.0034. Calculated for $C_{22}H_{16}O_3$ is 328.1099.

(±)-3β,4α-dihydroxy-1β,2β-epoxy-1,2,3,4-tetrahydrobenzo(g)-chrysene (isomer 2) (XXVII) (mixture XXVII and XXV)

In a 10ml round bottomed flask equipped with magnetic stir bar was placed 13.3mg (0.0426mmole) of dihydrodiol XXIII and 1.0ml tetrahydrofuran (THF). The flask was placed in a cold temperature bath and cooled to 10°C. *Meta*-chloroperoxybenzoic acid (73.3mg, 0.426mmole) was added and stirring was begun. Reaction progress was followed using ultraviolet spectroscopy. This was judged by disappearance of the dihydrodiol XXIII absorption at $\lambda=343$nm. After one hour the reaction mixture was diluted with 5ml ether and then washed with 5ml each of water, 2% sodium hydroxide (2x) and water.

The solution was dried using sodium sulfate and the solvent was removed under reduced pressure. The residue was dissolved in 300ul of anhydrous tetrahydrofuran for high performance liquid chromatography. Fractions, 25ul, were injected with a flow rate of 4 ml/minute. The solvent system was 40% THF in hexane and epoxide retention was 7.5 minutes. Best results were obtained when the HPLC column was cooled using circulating cold water. Some peak trailing was also observed and this material was not collected. Injections were repeated every 18 minutes. A total yield of 7.0mg of epoxides were obtained. The NMR spectrum indicates a 84:16 ratio of diol epoxide XXVII over XXV. NMR data (excluding diol epoxide XXV peaks): 9.27(d, J=8.0Hz, 1H), 8.70-8.86(m, 2H), 8.38-8.43(m, 1H), 8.05(m, 2H), 7.77(m, 4H), 5.66(m, $J_{3,4}=3.1$, $J_{4,OH}=4.4$Hz, H_4), 4.75(m, $J_{3,4}=3.1$, $J_{2,3}=6.0$, $J_{3,OH}=8.3$Hz, H_3), 4.51 (d, $J_{1,2}=4.0$Hz, H_1), 4.45(dd, $J_{2,3}=6.0$, $J_{1,2}=4.0$Hz, H_2), 2.86(d, $J_{3,OH}=8.3$Hz, OH_3), 1.90(d, $J_{4,OH}=4.4$Hz, OH_4). High resolution mass spectrum m/e 328.1083±.0023. Calculated value for $C_{22}H_{16}O_3$ is 328.1099.

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SYNTHESIS OF: 5,6-EPOXY-5,6,7,8-TETRAHYDROBENZO(G)-CHRYSENE; TRANS-5,6-DIHYDROXY-5,6-DIHYDRO BENZO(G)CHRYSENE; AND 5,6,7,8-BENZO RING DERIVATIVES OF BENZO(G)CHRYSENE

CHAPTER 3

INTRODUCTION

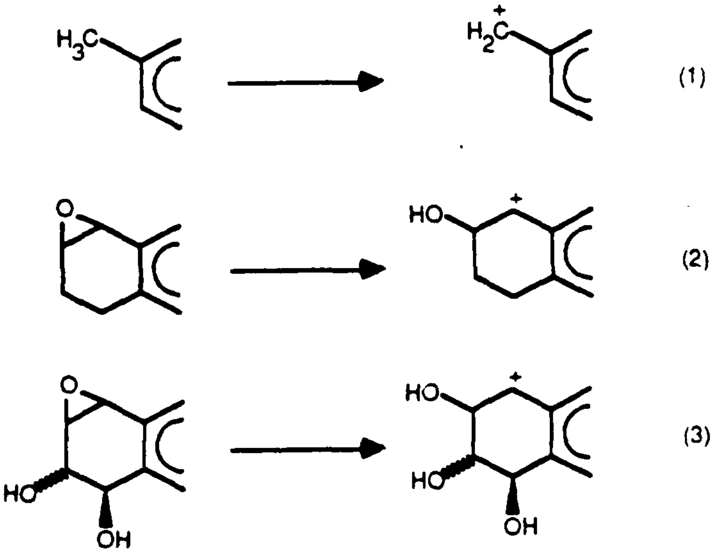
As mentioned earlier, polycyclic aromatic hydrocarbons (PAH), are found as contaminants of our atmosphere, by-products of energy production, particulates in tobacco smoke and components of other sources of environmental pollutants. Many of these PAH have been found to be carcinogenic and studies were implemented to determine what structural factors cause such uncontrolled cellular growth, what is a plausible mechanism, and what is the source of this activity (1).

The discovery that the bay region diol epoxide of benzo(a)pyrene was the ultimate carcinoginic metabolite paved the way for many additional studies on mechanism and structural features (1). In particular, an important advance in structure activity relationships is known as the "bay-region theory" (see chapter 1). Since the bay region theory uses simple mathematical methods of the perturbational molecular orbital theory, it is relatively easy to calculate a delocalization energy (ΔE_{deloc}) for a wide variety of alternant hydrocarbon systems.

The perturbational molecular orbital method neglects effects the trans-dihydroxyls may have in body metabolism. In fact, the calculations performed for the bay region theory for a given PAH apply equally to any of the three reactions shown in Figure 3.1 with the most rigorous being 1 and 2 (2).

Figure 3.1 (2)

Perturbational Molecular Orbital Processes



An excellent correlation (up to 92%) exists in the relationship between mutagenic activity and tetrahydro epoxide ΔE_{deloc} values calculated by perturbational molecular orbital method (2). Of particular interest are the results of benzo(e)pyrene tetrahydro-9,10-epoxide mutagenicity testing with strains of Salmonella typhimurium.

The mutagenicity values for the benzo(e)pyrene tetrahydro-9,10-epoxide were equivalent with those of benz(a)anthracene tetrahydroepoxide and slightly less than those for benzo(a)pyrene-9,10-epoxide, a potent mutagen. However, the bay region diol epoxides of benzo(e)pyrene are only slightly carcinogenic (2). Original theories indicated that the bay region diol epoxides would be biologically active if they were metabolically formed and that it is the lack of their formation that leads to the weak activity. Later results have indicated the diaxial conformation of the vicinal hydroxyls as an important factor in attenuating the carcinogenic activity of the benzo(e)pyrene bay region diol epoxides.

The multiple bay regions found in benzo(g)chrysene provide further opportunity to test mutagenicity and ΔE_{deloc} relationships. In addition, the synthetic route described in this chapter provides an opportunity to examine tumorigenicity of the 7,8-dihydro and the 5,6,7,8-tetrahydro-5,6-epoxide of benzo(g)chrysene.

As mentioned earlier, the mechanism of metabolism for benzo(a)pyrene has received much attention. The pathway has been established as hydrocarbon $\longrightarrow$ arene oxide $\longrightarrow$ dihydrodiol $\longrightarrow$ diol epoxide (Figure 3.3).

Figure 3.2

Benzo(g)chrysene Numbering Scheme

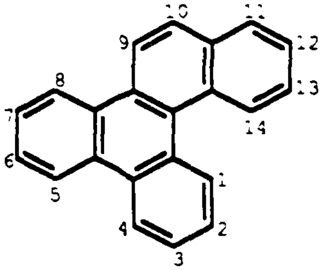
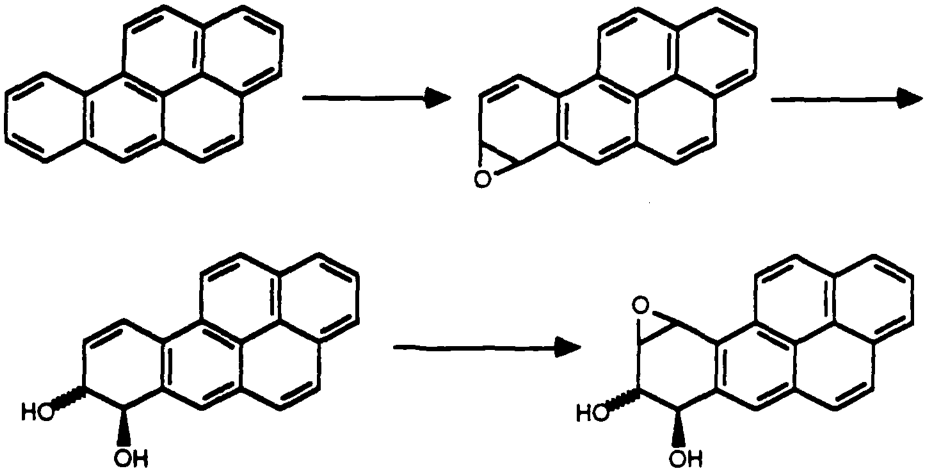


Figure 3.3 (3)

Metabolism of Benzo(a)pyrene



Early work on benzo(a)pyrene indicated three dihydro diols were products of metabolism and that the 7,8-dihydroxy-7,8-dihydrobenzo(a)pyrene, when further metabolized, binds ten times more effectively to DNA than other dihydrodiols. In 1977, research expanded to dihydrodiols of anthracene, phenanthrene, and benz(a)anthracene to determine which positional isomers of dihydrodiols can be metabolically activated to potent mutagens (1).

In addition to synthesizing the 5,6-tetrahydro epoxide, this work includes a synthesis of trans-5,6-dihydroxy-5,6-dihydro-benzo(g)chrysene. This will serve two purposes. The dihydrodiol will be available for mutagenicity tests as well as possible further metabolic studies. Furthermore, it provides authentic material that can be used as a marker when benzo(g)chrysene is studied under metabolic conditions.

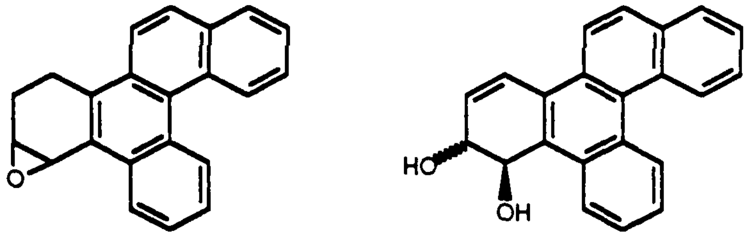
RESULTS AND DISCUSSION

The target molecules in this synthetic sequence are derivatives of benzo(g)chrysene (Figure 3.4). Functionality is introduced at the 5,6,7,8 positions of the PAH.

Figure 3.4

5,6,7,8-Benzo Ring Derivatives of

Benzo(g)chrysene



Benzo(g)chrysene has received recent attention in the study of theory and mechanism associated with PAH carcinogenesis. In particular, there is interest in how the vicinal dihydroxyl arrangement of PAH metabolites affects mutagenicity and tumorigenicity. With its 2 bay and 1 fjord regions, benzo(g)chrysene should provide several different steric environments that should lead to different conformational arrangements of the vicinal hydroxyl groups. Furthermore, there is a structural similarity between

5,6,7,8-tetrahydrobenzo(g)chrysene ring derivatives and those of benzo(e)pyrene.

Figure 3.5

Comparison Between Benzo Ring Locations

Benzo(g)chrysene and Benzo(e)pyrene

5,6,7,8-tetrahydrobenzo(g)chrysene

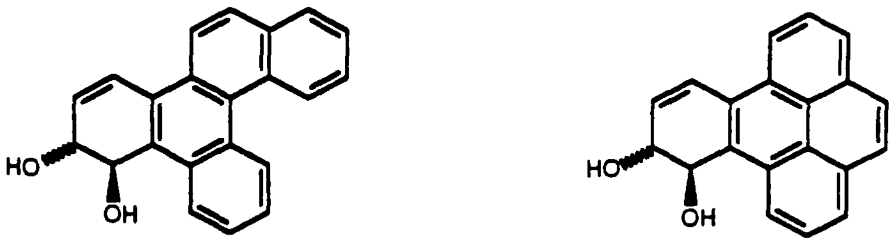
9,10,11,12-tetrahydrobenzo(e)pyrene

One would anticipate 5,6-epoxy-5,6,7,8-tetrahydrobenzo(g)chrysene to be slightly less mutagenic. This is based on $\Delta E_{deloc}/\beta$ at carbon 5 of 0.667 for benzo(g)chrysene and at carbon 9 of the 9,10,11,12-tetrahydro-benzo(e)pyrene-9,10-epoxide of .714. It appears direct comparison is also possible with the dihydro diols. Based on steric features of the benzo(g)chrysene system, the

Figure 3.6

Comparison Between Dihydro Diols

Benzo(g)chrysene and Benzo(e)pyrene

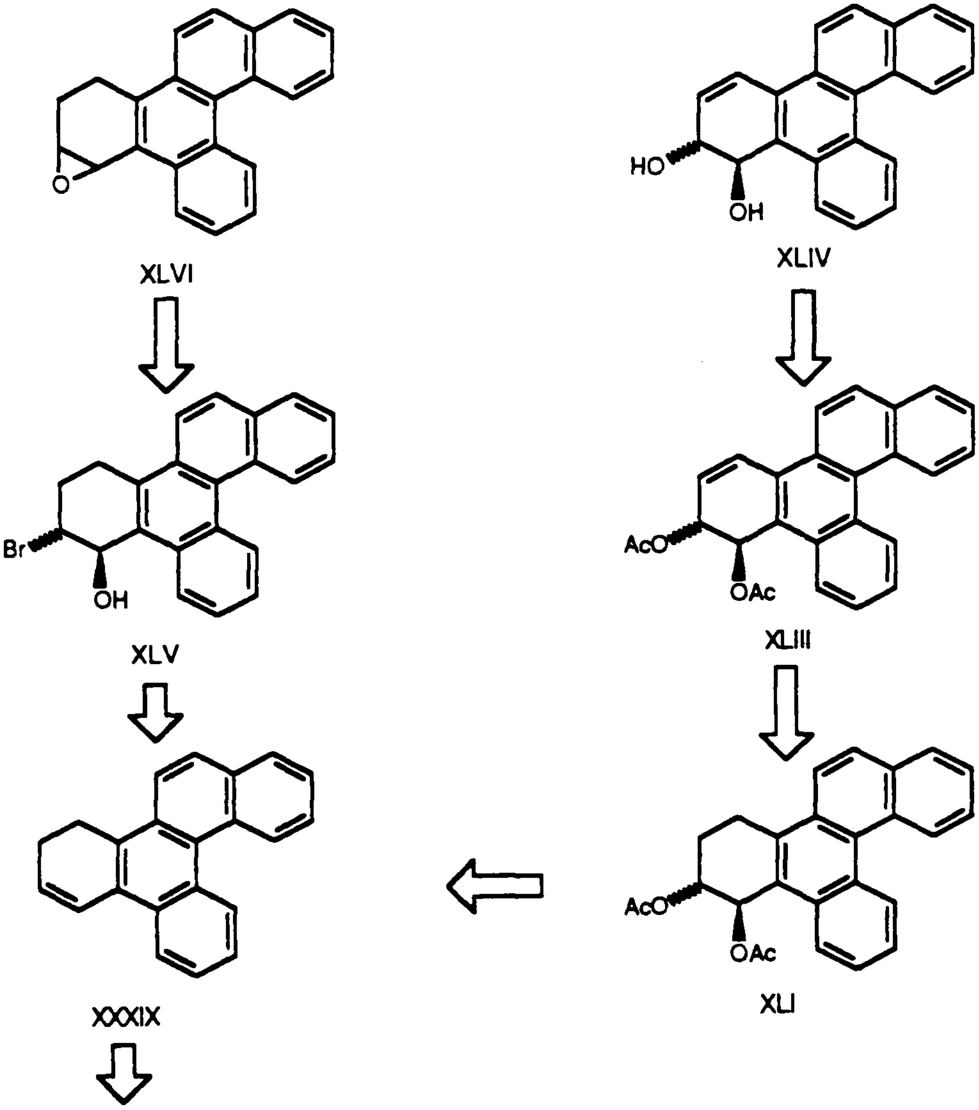


5,6-dihydroxy-5,6-dihydrobenzo(g)chrysene

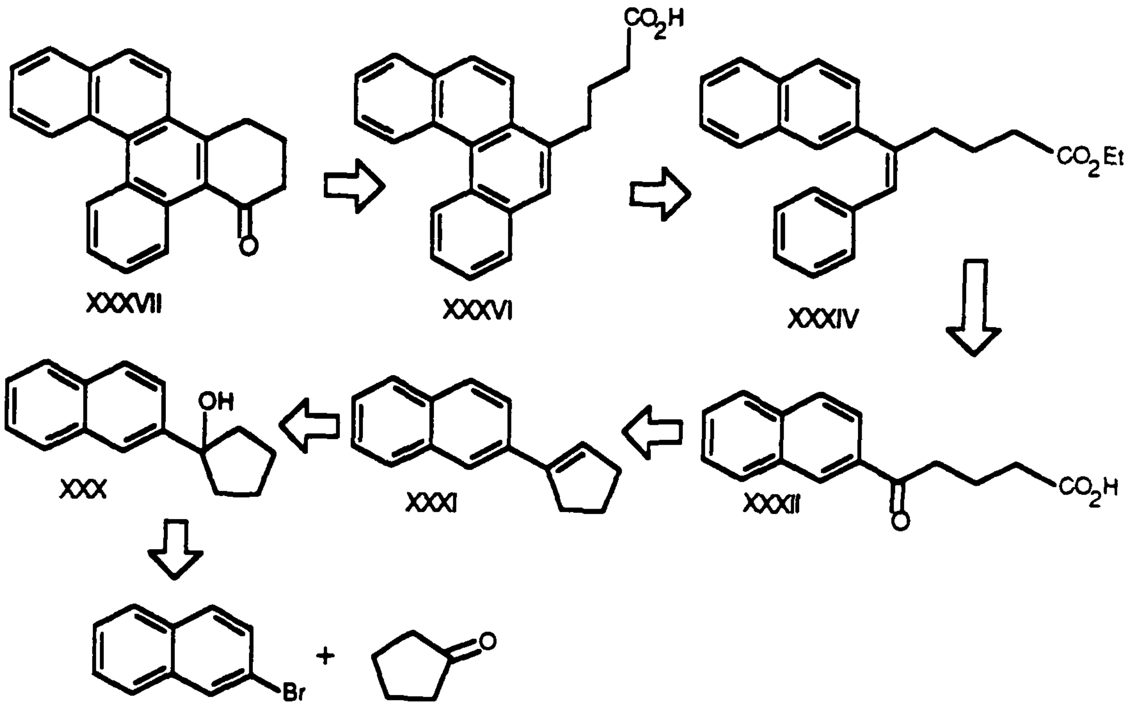
9,10-dihydroxy-9,10-dihydrobenzo(e)pyrene vicinal hydroxyls most probably adopt a quasi diaxial conformation since the benzylic hydroxyl (C_5) forms part of a bay region. That structural feature is very similar to that of benzo(e)pyrene-9,10-dihydrodiol which is known to exist in the diaxial conformation (4).

A retro synthetic approach to the target molecules is shown in Scheme 3.1. Both the tetrahydroepoxide (XLVI) and the dihydrodiol (XLIV) are available from 7,8-dihydrobenzo(g)chrysene (XXXIX).

Scheme 3.1



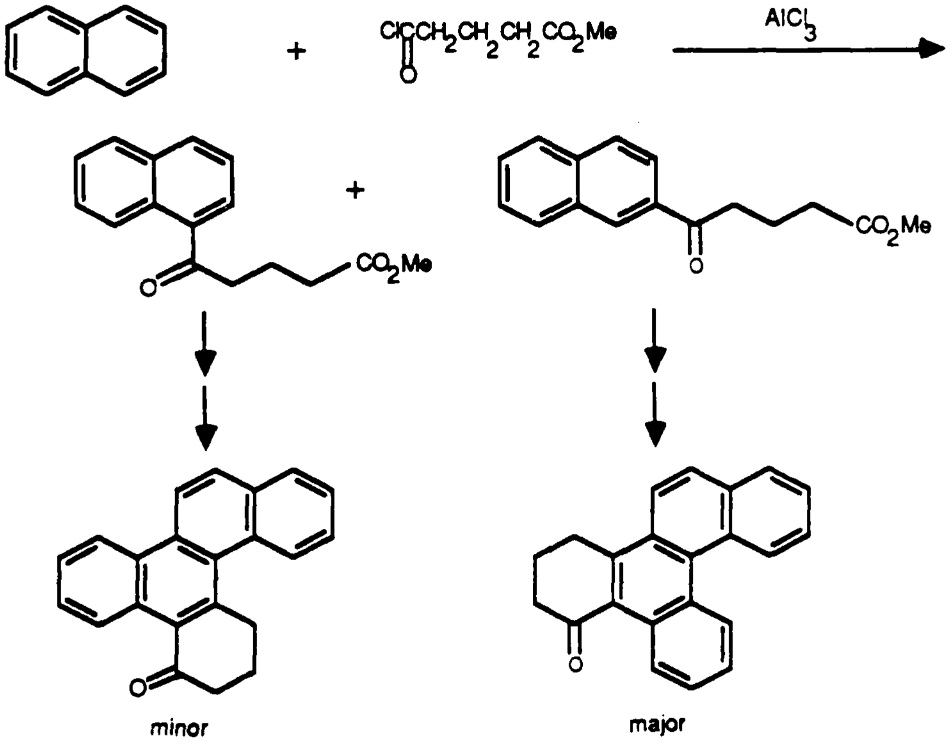
Scheme 3.1 (continued)



The retro synthetic scheme as shown was chosen to permit the use of as many known successful reactions as possible and to ensure regiospecific reactions. For this scheme, the starting materials 2-bromonaphthalene and cyclopentanone are commercially available and were used without further purification.

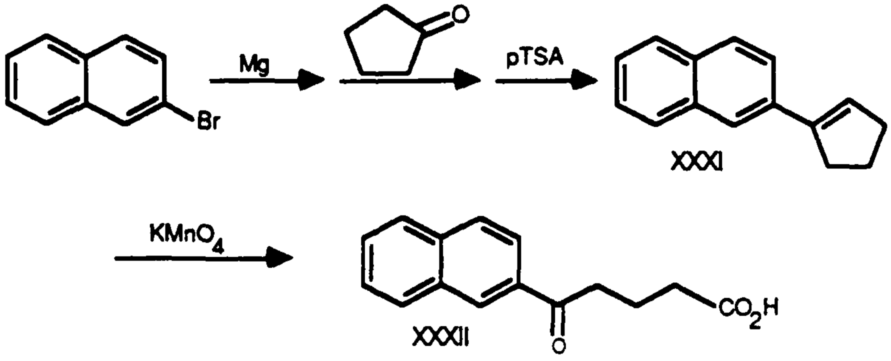
The method used here follows quite closely the preparation of the 1,2,3,4-tetrahydrobenzo(g)chrysene derivatives already described. However, the general approach to the 5-oxo derivative (XXXVII) was discovered quite by accident. In an early experiment we attempted to react naphthalene with methyl-4-(chloroformyl) butyrate and $AlCl_3$ in a Friedel-Crafts acylation. According to literature reports (5) one would anticipate forming only the 1-substituted naphthalene. However, when the product of this reaction was carried through to the ketone, the major product isolated was the 5-oxo-5,6,7,8-tetrahydro benzo(g)chrysene and not the expected 4-oxo-1,2,3,4-tetrahydro benzo(g)chrysene.

Scheme 3.2



Cyclization of the 1-substituted product should produce the 4-oxo-benzo(g)chrysene while the 2-substituted naphthalene would produce the 5-oxo compound XXXVII. This in no way implies that the major product of the Friedel-Crafts acylation is the 2-naphthyl derivative. However, the methods of work up and attempts to crystallize the products (the 1-naphthyl derivative is now known to be an oil while the 2-naphthyl product is a solid) resulted in what appeared to be failure to prepare 4-oxo-1,2,3,4-tetrahydro benzo(g)chrysene. The synthetic sequence shown in Scheme 3.1 provides an unambiguous product that was later shown to be identical with the solid product from the Friedel-Crafts procedure, after conversion to the methyl ester.

Scheme 3.3



As the retro synthetic scheme indicates, a key process in this synthesis is the unambiguous preparation of 4-(2-naphthoyl)-butyric acid (XXXII). We learned earlier in this project that a variety of reagents and conditions were available to carry out oxidative cleavage of a benzylic alcohol on a cycloalkane or a cycloalkene such as XXXI to form keto acids. With this earlier experience (chapter 2), we elected to perform oxidative cleavage on the cycloalkene.

(2-Naphthyl)-1-cyclopentanol was prepared by Grignard reaction with cyclopentanone. Preparation of the Grignard reagent was attempted using both anhydrous ethyl ether and anhydrous tetrahydrofuran (THF). We had little success with ethyl ether, while THF provided good yields of alcohol. However, even after the Grignard reagent started forming, the reaction would not sustain a reflux and external heat was required to maintain reaction progress. Cyclopentanone was added dropwise while cooling and then the mixture was refluxed for two hours. The magnesium salt was hydrolyzed the next day after stirring at room temperature.

Instead of isolating the alcohol at this point, we elected to take the entire crude product on to the cycloalkene (6). Solvent was removed, and the residue was dissolved in benzene and refluxed after adding a few crystals of para-toluenesulfonic acid. Reaction progress was easily monitored by thin layer chromatography (TLC) with benzene as the solvent. As the cycloalkene formed, a less polar spot began forming on TLC. After two hours the reaction was complete. After purification by flash chromatography, XXXI was available in 85.9% yield based on magnesium. The NMR spectrum of XXXI is shown in Figure 3.7.

Oxidative cleavage of XXXI to give keto acid XXXII

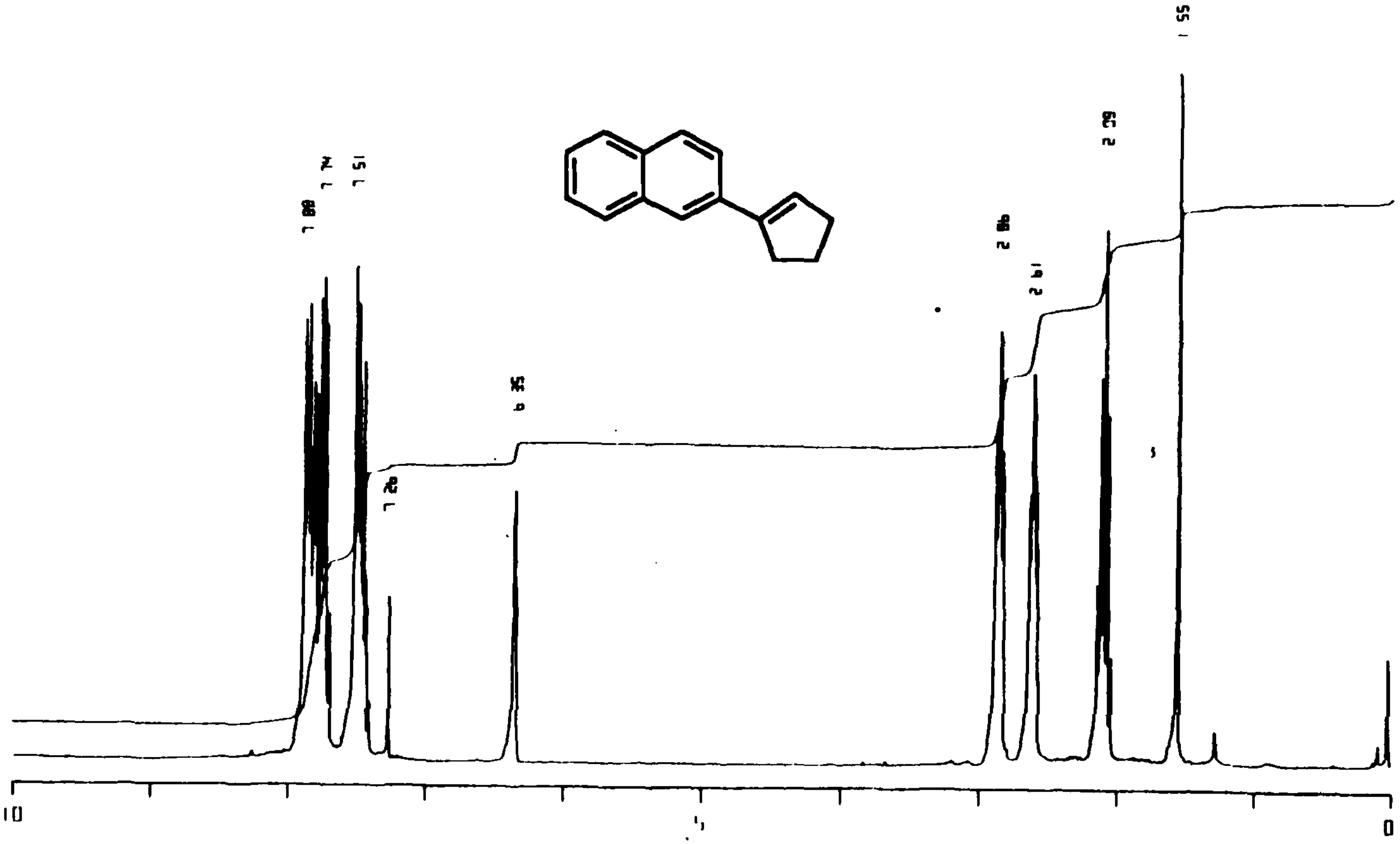


Figure 3.7 300 MHz NMR Spectrum ($CDCl_3$) of XXXI

wasn't without its own challenge. Initially a small sample (97mg) of XXXI was oxidized using potassium permanganate and 18-crown-6 ether (7). The yield of keto acid (57%) was respectable, especially when compared with earlier results of oxidative cleavage when cyclopentene was at the 1-naphthyl position. Unfortunately, this method didn't seem suited to larger scale reactions (see Table 3.1). The major problem again appears to be the manganese (IV) oxide forming on the surface of potassium permanganate. After stirring overnight, the reaction was only 50% complete. Ten percent excess permanganate was then added and three hours later another five percent (4.00g) permanganate was added and again after 1.5 hours. This went overnight again and a variety of over oxidized products resulted.

Table 3.1

Oxidative Cleavage to Form 1-(2-Naphthoyl)-Butyric Acid

g XXXI	g XXXII*	Conditions	%Yield
0.097	0.069	2hr, R.T.	57
5.578	2.77**	24hr	39(ester)
0.970	0.679	16 hr, mech. stirring	56
0.970	0.528	sonicator	43
0.970	0.7192	glass beads	59
0.970	0.5693	glass beads	47
1.1287	0.7839	glass beads	56

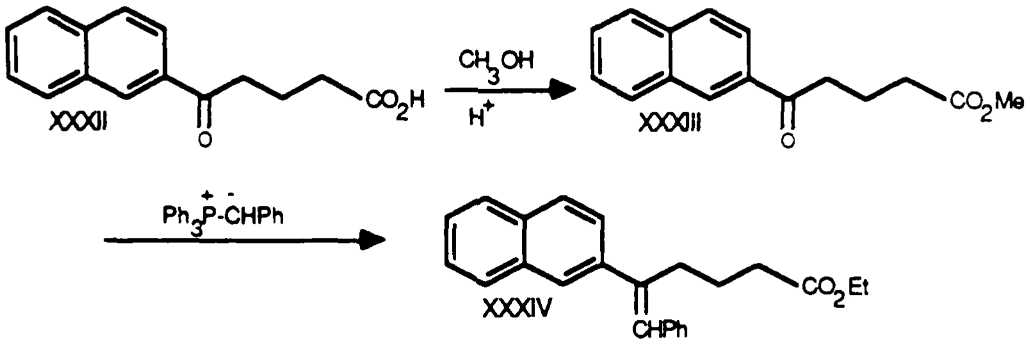
*purity estimated by NMR.

**Excess $KMmO_4$

Reaction size was markedly reduced and mechanical (overhead) stirring added. This seemed to improve the reaction, yet it required overnight stirring and product purity was approximately 85% by NMR. Similar results were obtained when the reaction was run in a sonicator with overhead stirring.

However, we were able to get repeatable results when glass beads were added to the flask. Overhead stirring then "grinds" the solid residue in the bottom of the flask and apparently removes sufficient oxide coating from the permanganate. The reaction proceeded smoothly and in 4 hours gave a 56% yield of keto acid XXXVII.

Scheme 3.4



Conversion of the keto acid to keto ester went smoothly by refluxing XXXII in methanol with acid catalyst. The NMR spectrum of XXXIII is shown in Figure 3.8. The Wittig reagent was again prepared using "super dry" ethanol that was dried over magnesium ethoxide. Formation of stilbene XXXIV however was very slow. This reaction rate is

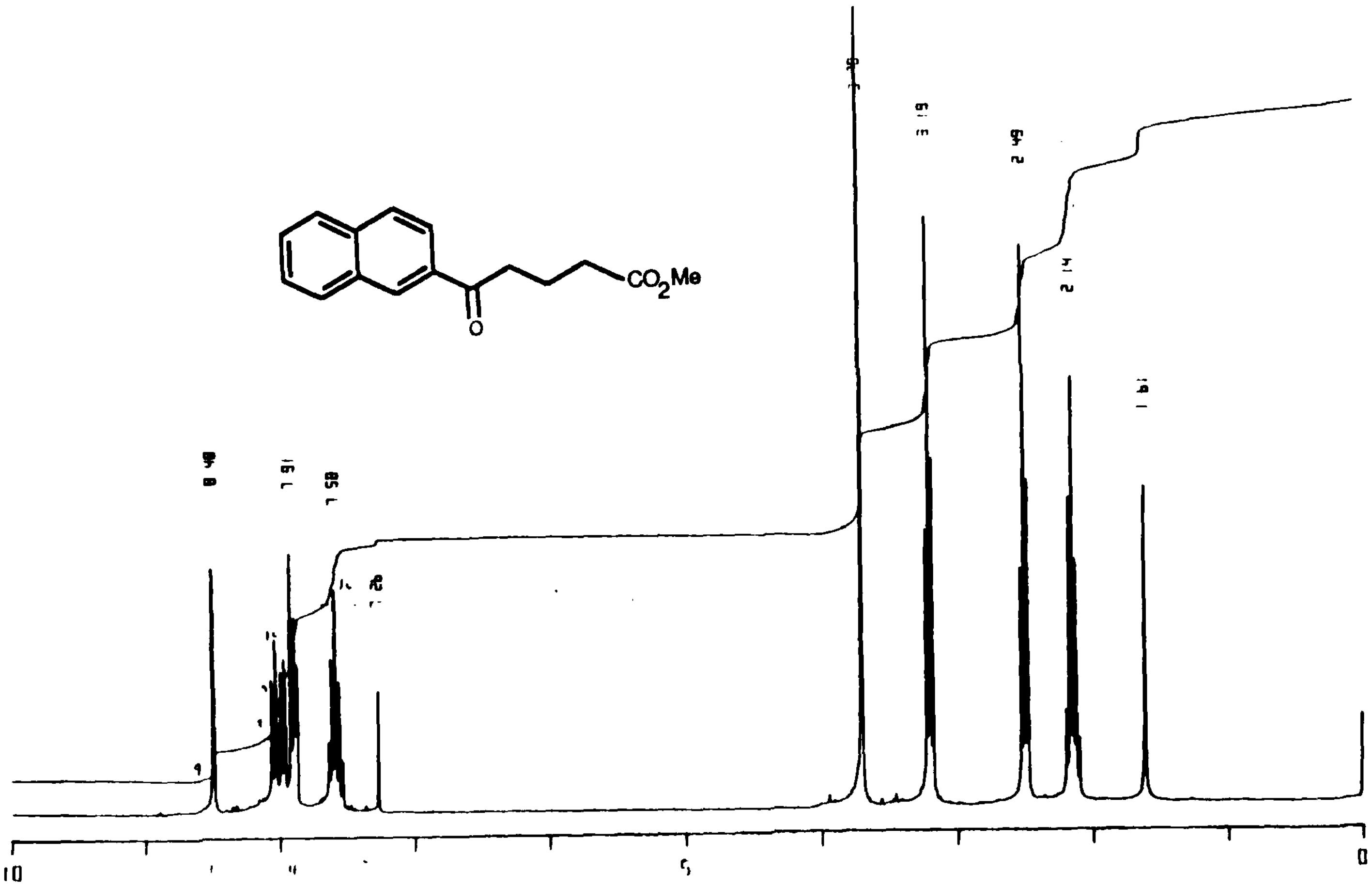
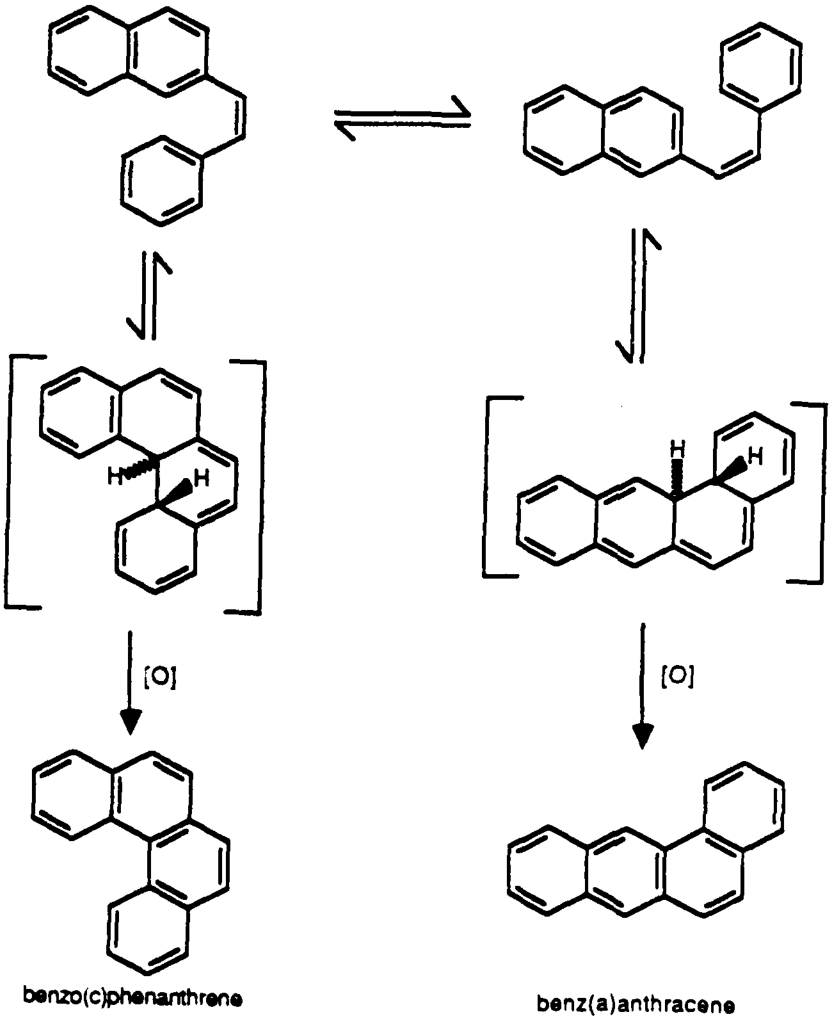


Figure 3.8 300 MHz NMR Spectrum ($CDCl_3$) of XXXIII

reflected in the ratio of products generated during the Wittig reaction. Slower reactions tend to favor *E*-isomer formation and this is also true in this case.

The reaction was complete after 16 hours of reflux and an NMR spectrum of the product indicates 56% *E* versus 44% *Z*. The assignment of the olefinic *E* proton to δ=6.90ppm and the *Z*-proton to δ=6.56ppm in the NMR spectrum (not shown) is based on proton predictions using

Scheme 3.5



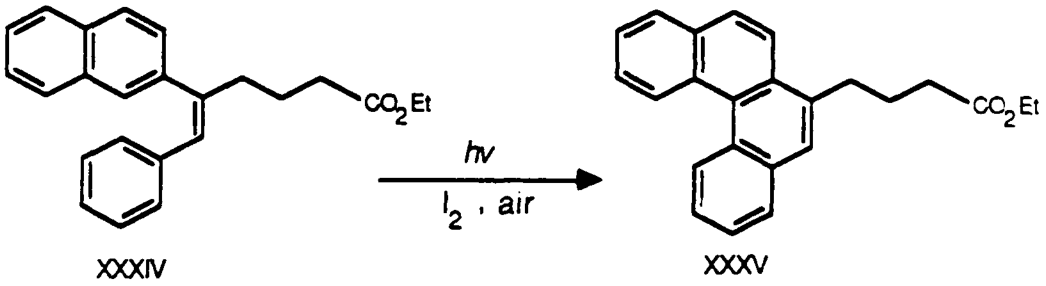
Silverstein, Bassler, and Morrill (8) values. Although the *Z* isomer is more desired, we were not unhappy with the product mixture as there is known to be an equilibrium between the *E* and *Z* forms during photolysis (9).

There are two sites available to obtain photocyclized products. Scheme 3.5 illustrates the routes leading to different products. Either product could be expected from this photocyclization route; however, previous results indicate that benzo(c)phenanthrene is almost an exclusive product. Benz(a)anthracene has only been reported when the reaction takes place in high concentrations of iodine. No benz(a)anthracene is found when oxygen is used as the trapping reagent (9).

In 1977, Nagel, et. al., demonstrated that α-substituted 2-styryl naphthalenes will also photocyclize to substituted benzo(c)phenanthrenes. However, substitution was limited to methyl derivatives (10).

Our synthetic procedure uses this cyclization technique. In a pyrex vessel with air inlet tube and

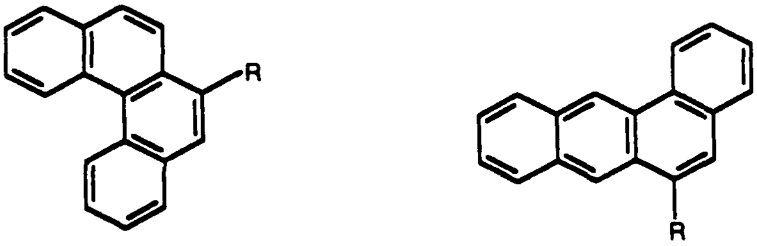
Scheme 3.6



condenser, we placed 2-styrylnaphthalene XXXIV using alkene-free cyclohexane as the solvent. After a small amount of iodine was added, the flask was irradiated at 300nm until an NMR aliquot indicated approximately 90% reaction. This usually took in excess of 40 hours.

Proton NMR of the product gave a downfield multiplet at 9.07ppm(2H), a doublet at 8.10(1H) and multiplets at 8.02(1H), 7.96(2H), and 7.62(4H). The only singlet occurs at 7.74ppm (1H).

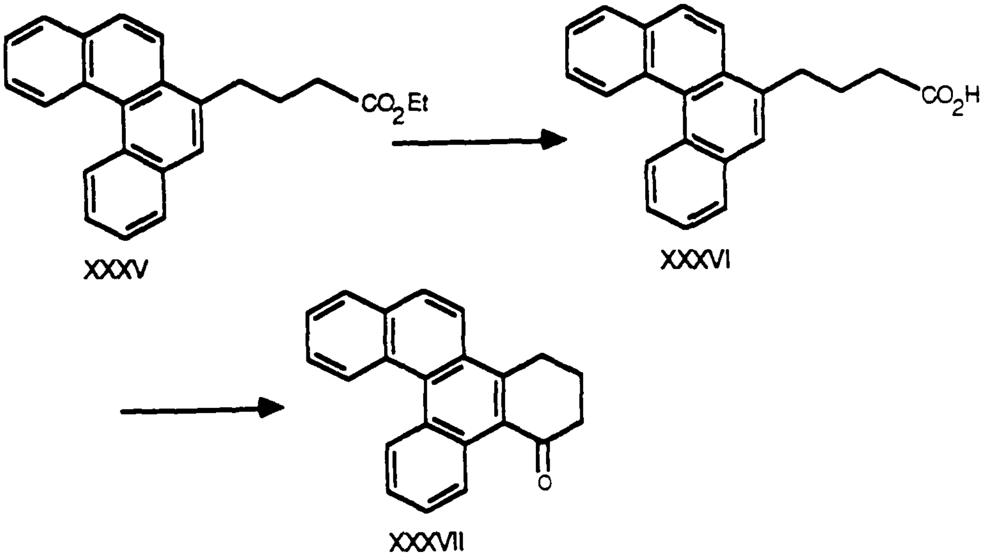
Scheme 3.7



Benz(a)anthracene is easily ruled out as a product of this reaction. One would anticipate two downfield singlets at approximately 8.2 and 9.0 for protons 7 and 12 respectively. Since these signals are absent, the product cannot be the benz(a)anthracene.

Benzo(c)phenanthrene proton chemical shifts have recently appeared in the literature (11). Downfield protons occur at 9.14ppm(2H), 8.02(2H) and 7.90(2H). Additional signals are at 7.83(2H), 7.68(2H) and 7.62(2H). This information, coupled with the fact that there is a singlet that corresponds to the proton at position 5, is consistent with benzo(c)phenanthrene structure XXXV.

Scheme 3.8



Ester XXXV is a thick yellow oil. It was isolated by solvent evaporation and flash chromatography and used without further purification.

The ester was easily hydrolyzed by refluxing in 10% aqueous potassium hydroxide solution. After it was acidified and extracted with methylene chloride, acid XXXVI was crystallized with ether/petroleum ether. The NMR spectrum for XXXVI is shown in Figure 3.9. The mass spectrum gave a molecular ion m/e 314 (Figure 3.10).

Cyclization of the acid to ketone XXXVII occurred with methanesulfonic acid. Acid XXXVI was placed in methanesulfonic acid and heated for 1.5 hours at 60°C. The mixture turned dark brown, yet after work up (see

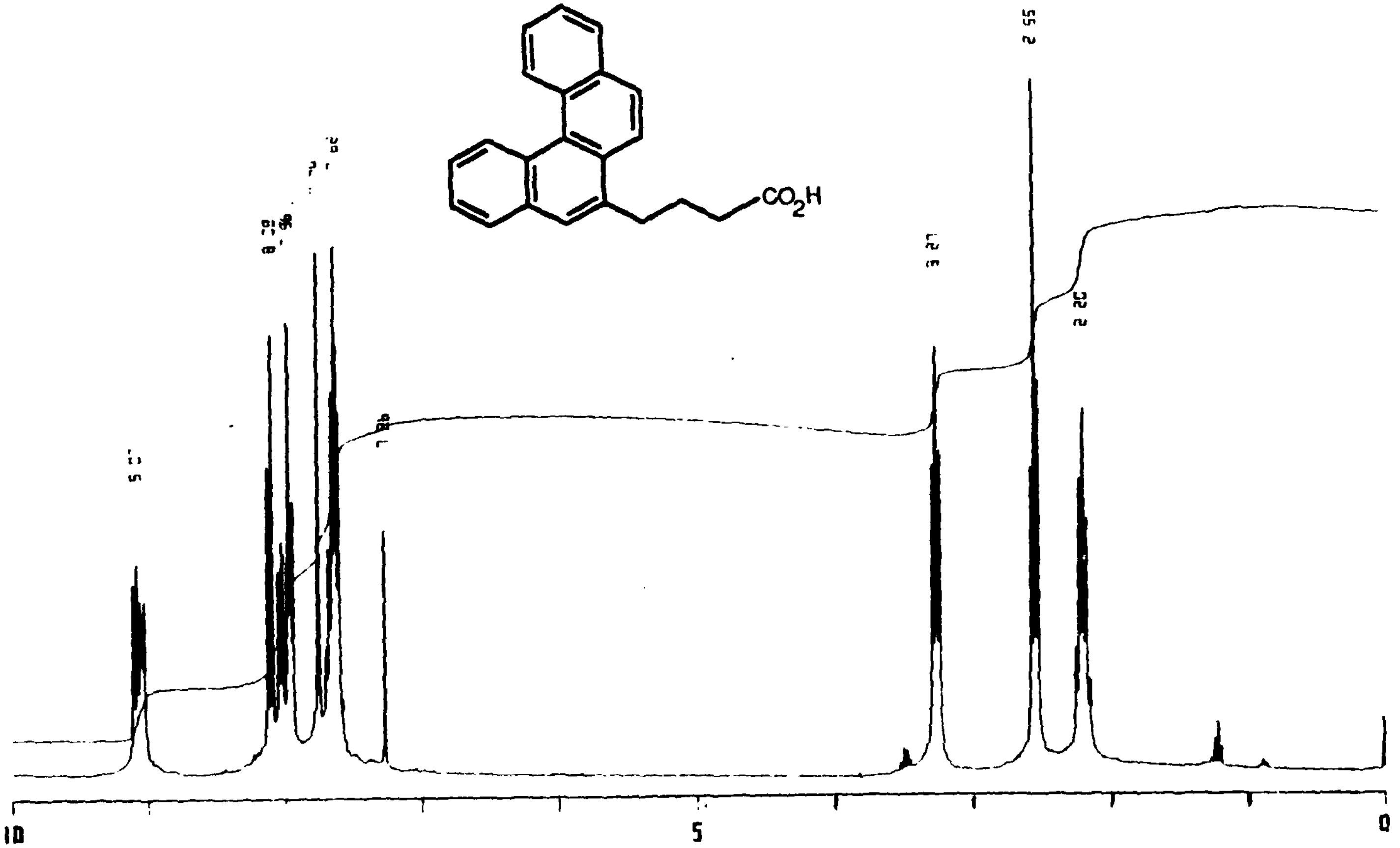


Figure 3.9 300 MHz NMR Spectrum (CDCl$_3$) of XXXVI

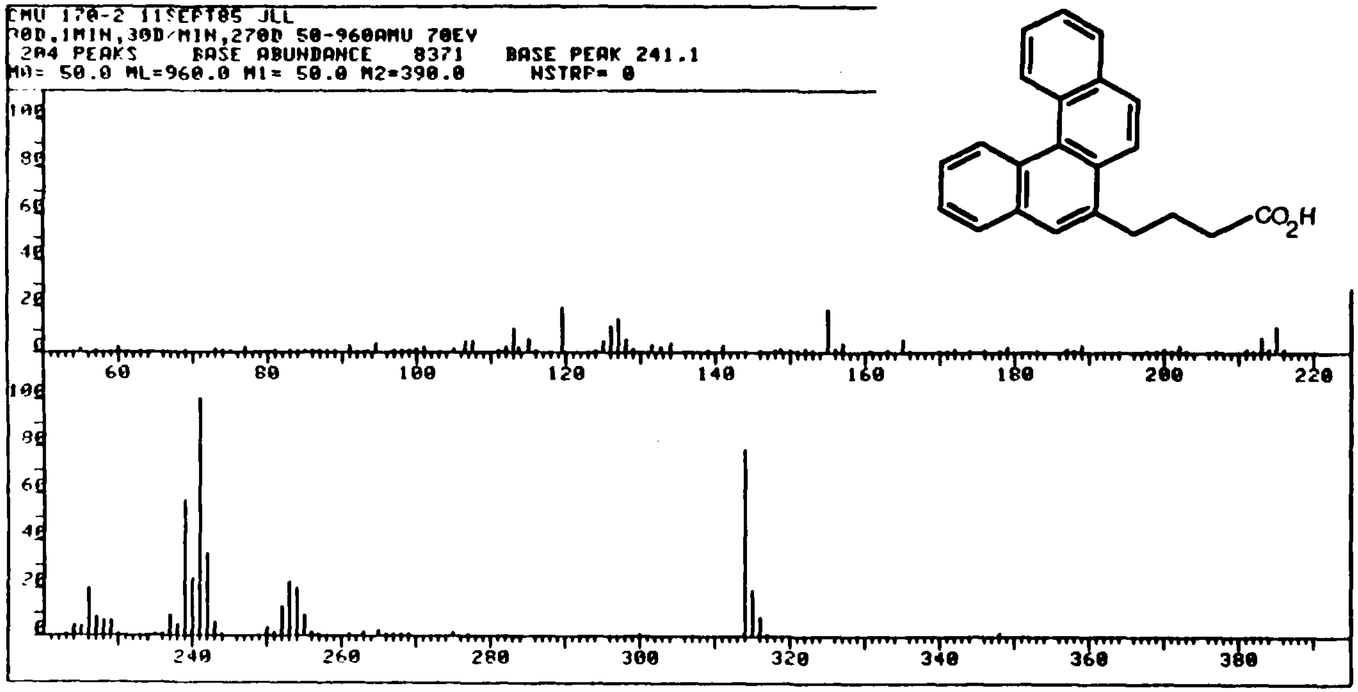


Figure 3.10 70 eV Mass Spectrum of XXXVI

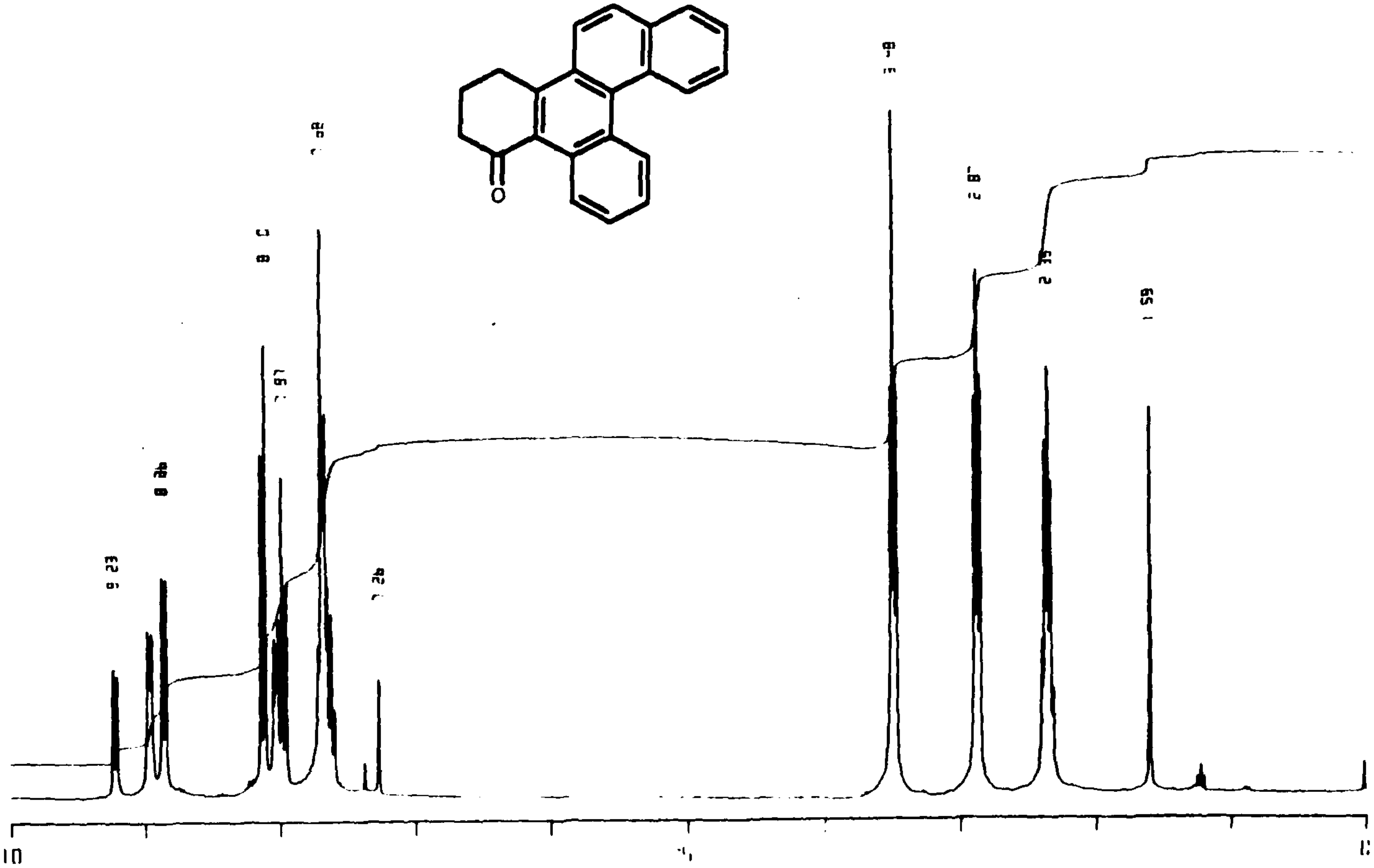


Figure 3.11 300 MHz NMR Spectrum ($CDCl_3$) of XXXVII

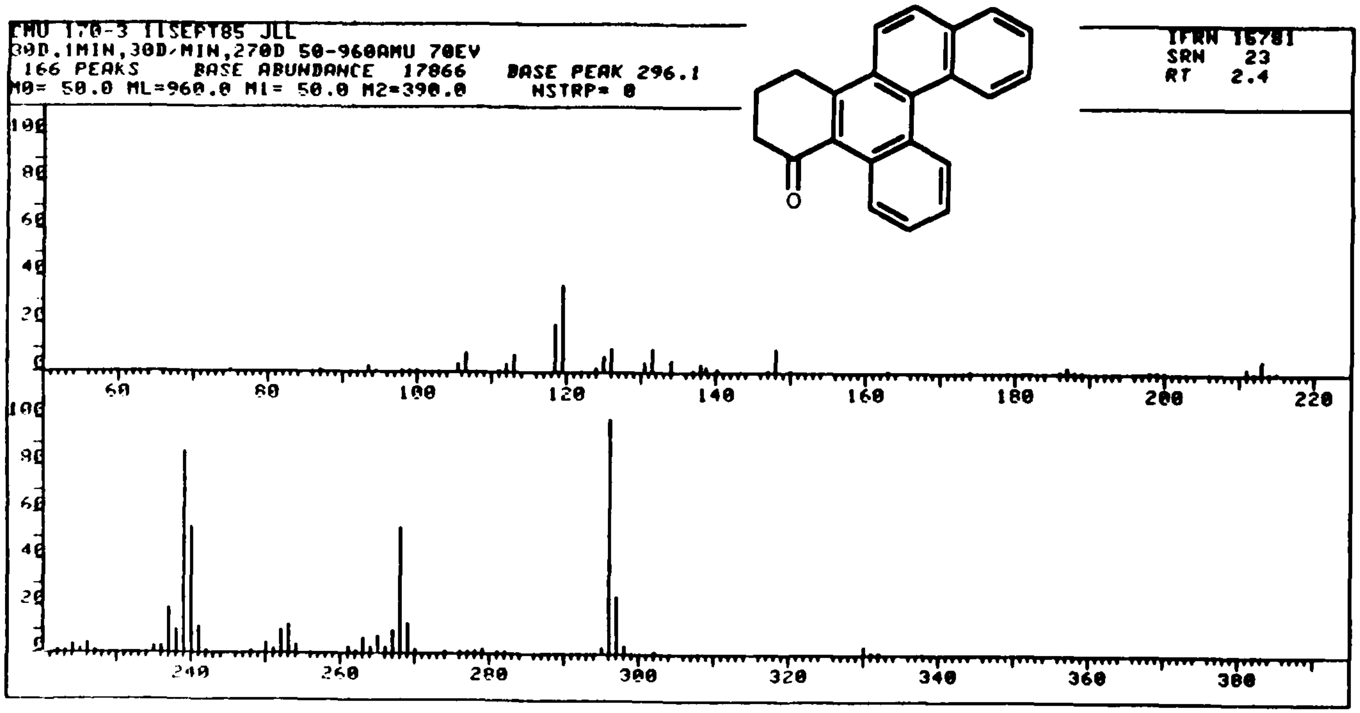


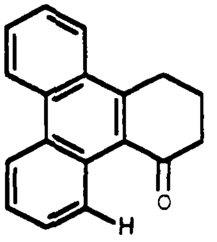
Figure 3.12 70 eV Mass Spectrum of XXXVII

Experimental), purification by flash chromatography, and crystallization with ether gave 86% yield of ketone XXXVII. The melting point was 138-140°C and the mass spectrum gave a molecular ion at m/e 296. The NMR spectrum gave a downfield doublet (J=7.8Hz) at 9.21ppm in addition to the expected fjord region protons H_{14} and H_1 (δ=8.96 and [illegible]) of benzo(g)chrysene. See Figures 3.11 and 3.12 for the NMR and mass spectra of XXXVII.

This downfield absorption is common among tetrahydro ketones with similar structures. An analogy exists with triphenylene ketone. An NMR spectrum of an authentic sample gave a downfield doublet at 9.24ppm which can be attributed to the indicated proton in Figure 3.13.

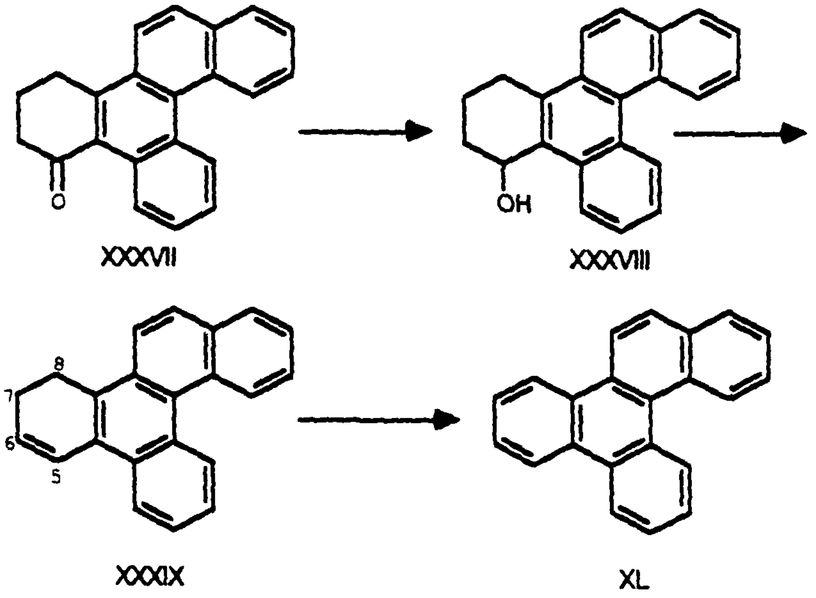
Figure 3.13

Triphenylene Ketone



Reduction of ketone XXXVII took place smoothly in methanol with sodium borohydride. The reaction mixture was stirred at room temperature for one hour. Water was added and then the alcohol, XXXVIII, was extracted with methylene chloride and the solvent evaporated leaving a white to cream colored solid with a melting point 158-162°C. Mass spectrum m/e 298, 280 ($-H_2O$).

Scheme 3.9



No further purification was used and the alcohol was dissolved in benzene. Dehydration occurred using a catalytic amount of para-toluenesulfonic acid. Dehydration wasn't as facile in this ring system as noted in the 1,2,3,4-benzo ring (Chapter 2). After stirring the reaction mixture at room temperature for 16 hours, alcohol still remained, as determined by thin layer chromatography (TLC). To drive the reaction to completion, the external temperature was raised to 50°C for 1 hour. TLC verified that dehydration was complete and the mixture was cooled, washed with water and the solvent was removed. Purification at this point was by flash chromatography.

Best results were obtained when the crude product was introduced onto the column with a very small amount of benzene. Since the dihydrobenzo(g)chrysene is very non-polar, it elutes readily with petroleum ether, and benzene must be held to a minimum or "polar material" will elute from the column in addition to the product. After the crude material was placed on the column, petroleum ether was used as the solvent to remove dihydro compound XXXIX.

After removing the solvent we were left with a light green oil that would not solidify even after storing several days at-20°C. The NMR spectrum (Figure 3.14) is consistent with the proposed structure. In addition to the two distinct protons (H_1 and H_{14}) at 9.02ppm, the other aromatic absorptions occur between 7.57 and 8.30. The olefin proton, H_6, gives a multiplet with coupling to H_7(J=4.8Hz) and to H_5(J=9.9Hz). The other alkene proton is hidden in the aromatic region and was located by homonuclear decoupling. When H_6 was irradiated, the doublet at 7.37 collapsed to a singlet and therefore could be assigned as H_5. Benzylic protons were found as a triplet (J=8.8Hz) at 3.34 and protons H_7 were found at 2.54ppm. The mass spectrum, Figure 3.15, gave a molecular ion, m/e 280.

At this point in the synthesis, a sample of benzo(g)chrysene was required for biological testing in another laboratory and as a marker in analytical procedures. In addition, preparation of benzo(g)chrysene from dihydro aromatic XXXIX also verifies the structure of XXXIX.

7,8-Dihydrobenzo(g)chrysene, XXXIX, was dissolved in dry benzene. 2,3-Dichloro-5,6-dicyano-1,4-benzoquinone, (DDQ), was added and the mixture was stirred at room

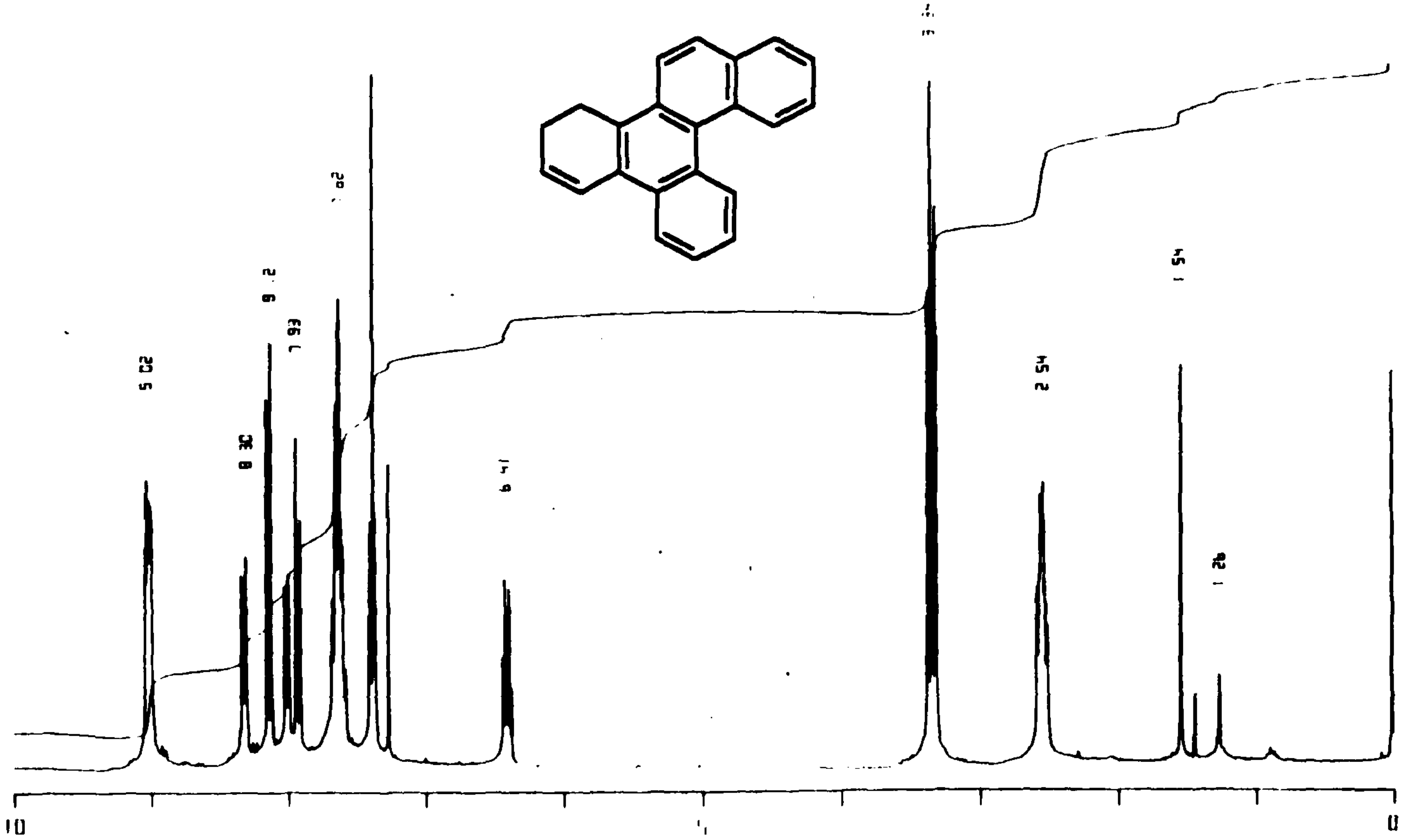


Figure 3.14 300 MHz NMR Spectrum ($CDCl_3$) of XXXIX

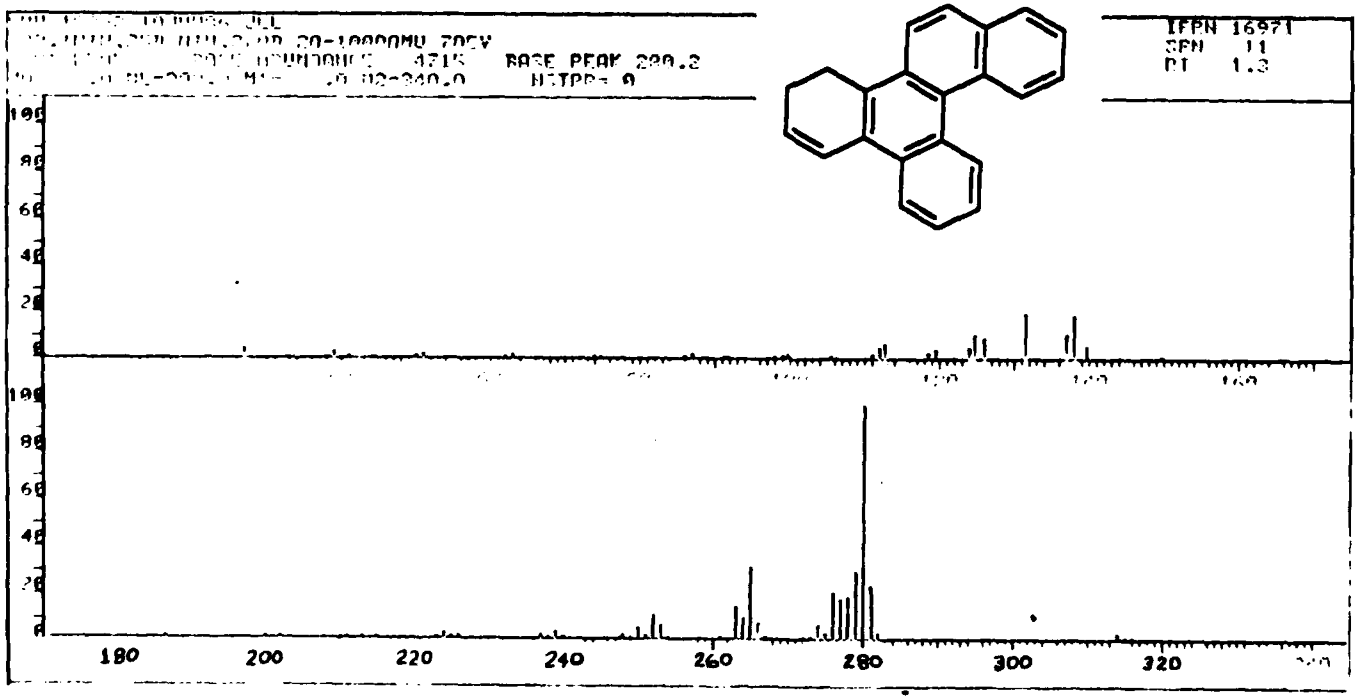


Figure 3.15 70 eV Mass Spectrum of XXXIX

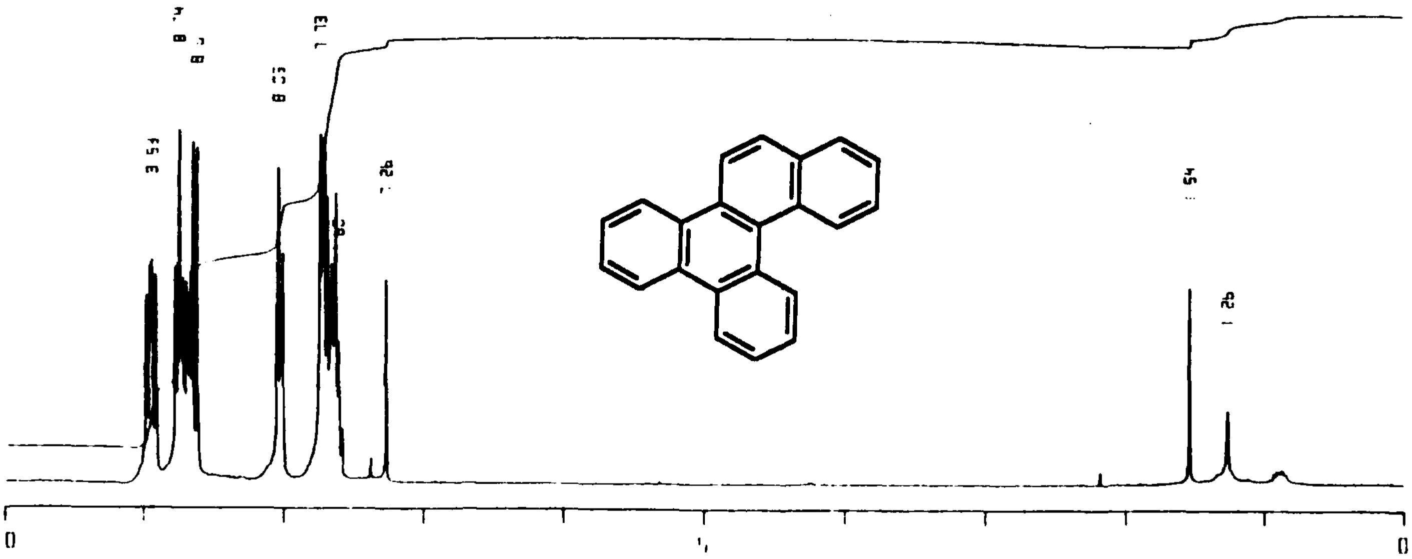


Figure 3.16 300 MHz NMR Spectrum ($CDCl_3$) of XL

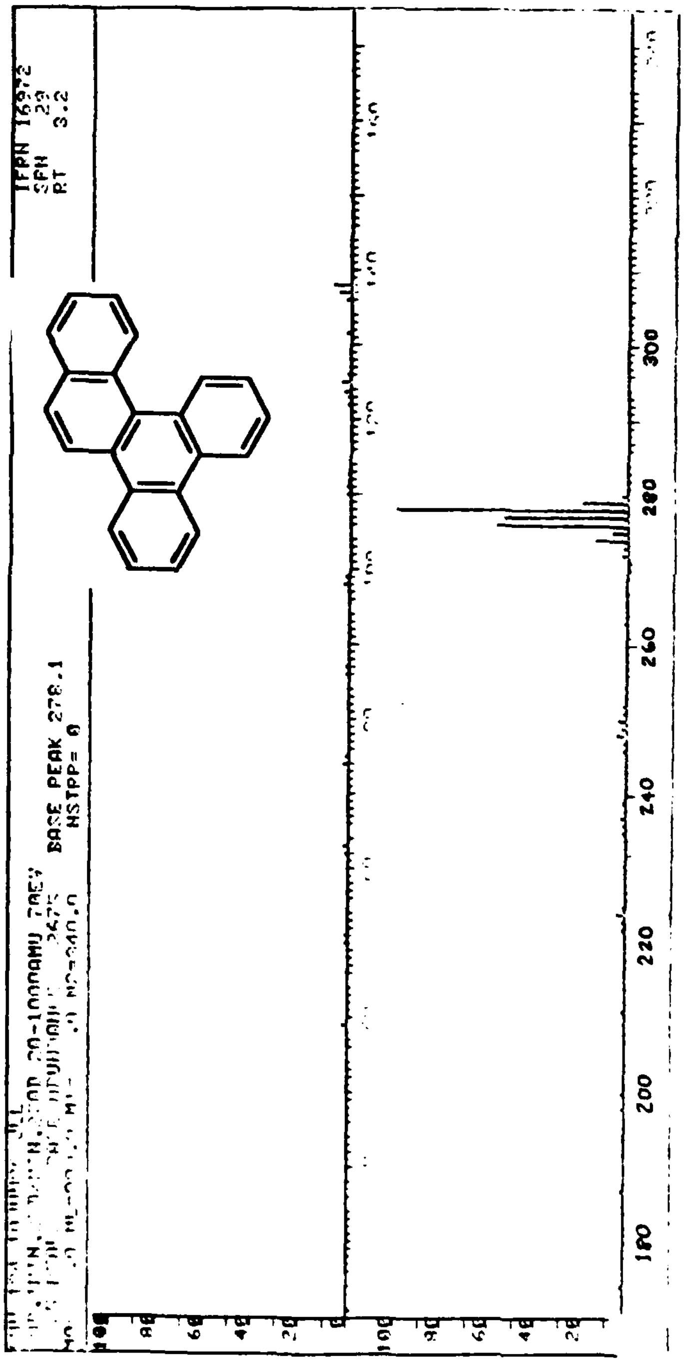


Figure 3.17 70 eV Mass Spectrum of XL

temperature. The reaction mixture turned a dark green color. After work up and purification by preparative thin layer chromatography, a white solid was collected with melting point 112-114°C. The NMR and mass spectra are shown in Figures 3.16 and 3.17. NMR data is tabulated in Table 4.2 along with literature values. Comparison between the two sets of values reveals excellent correlation. In addition, another sample of benzo(g)chrysene was prepared using 1,2-dihydrobenzo(g)chrysene from Chapter 2. The NMR spectrum obtained from that sample matched perfectly with that of the product of DDQ with XXXIX. The parent hydrocarbon ring structure was thus verified and the product of photocyclization, XXXV, was confirmed. We now had the core material for preparing the target dihydrodiol and tetrahydroepoxide.

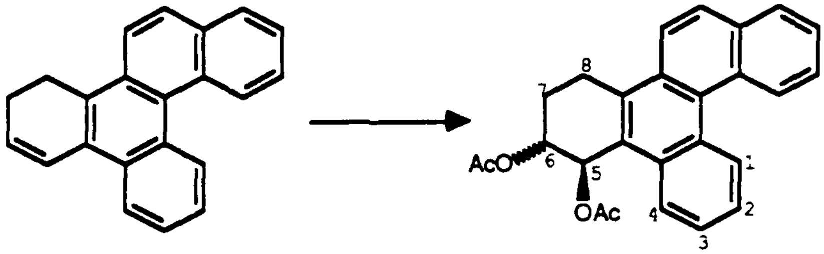
Table 3.2

Benzo(g)chrysene NMR Results

DDQ+XXXIX, 300MHz	Literature (11), 500MHz	
8.90-8.96(2H)	8.95(H_{14})	8.91(H_1)
8.60-8.76(4H)	8.72(H_4)	8.69(H_5)
	8.63(H_8)	8.58(H_9)
8.00-8.05(2H)	8.00(H_{11})	7.97(H_{10})
7.57-7.74(6H)	7.69(H_7)	7.63(H_6)
	7.65(H_3)	7.64(H_2)
	7.63(H_{13})	7.60(H_{12})

The Prevost reaction was used to form tetrahydro diacetate XLI. Silver acetate and iodine in dry benzene gave the initial iodo acetate in 30 to 45 minutes. However, conversion to the diacetate was much slower and required reflux for 20 hours. Reaction products were separated by flash chromatography giving an 60% yield of XLI and a minor, non-polar product. The NMR spectrum verified that this minor component (7%) was benzo(g)chrysene.

Scheme 3.10



XXXIX XLI

The NMR spectrum of the major component (Figure 3.18) is consistent with the structure shown in Scheme 3.10. The tetrahydrobenzo ring proton assignments were confirmed by homonuclear decoupling experiments. The benzylic acetoxy proton occurs at 6.69ppm with coupling constant of 3.3Hz. Because the Prévost reaction is known to give <u>trans</u> addition (12), this small coupling is indicative of diequatorial arrangement of the protons at C_5 and C_6 or diaxial conformation of the vicinal acetoxy groups. NMR results *(tetrahydrobenzo ring only):* 6.69(d, J=3.3Hz, H_5), 5.41(m, H_6), 3.28-3.43(m, $2H_8$), 2.17-2.44(m, $2H_7$), 2.12(s, 3H), 2.01(s, 3H). The mass spectrum provides a molecular ion at m/e 398 with loss of one and two equivalents of acetic acid, m/e 338, 278. (Figures 3.18 and 3.19.)

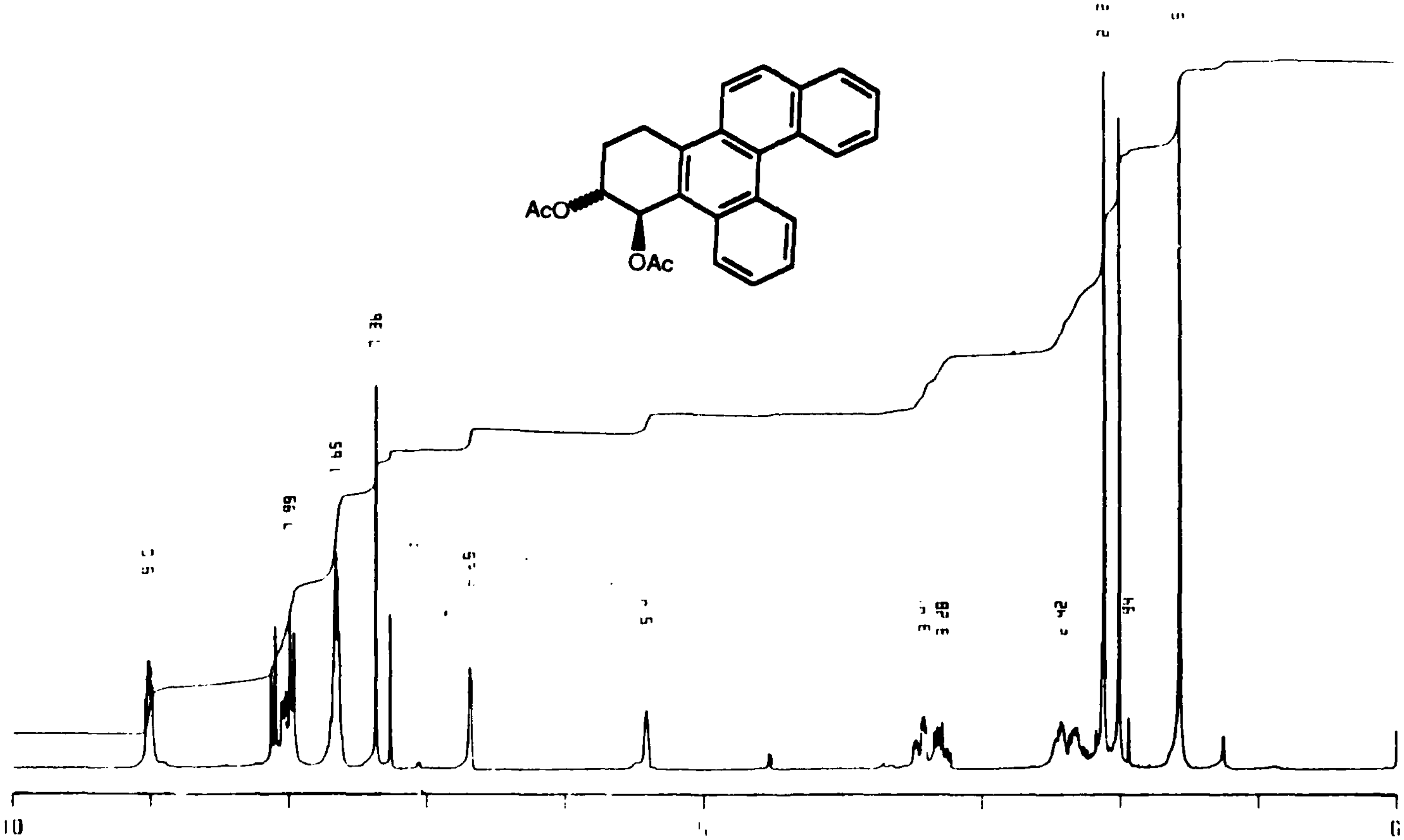


Figure 3.18 300 MHz NMR Spectrum ($CDCl_3$) of XLI

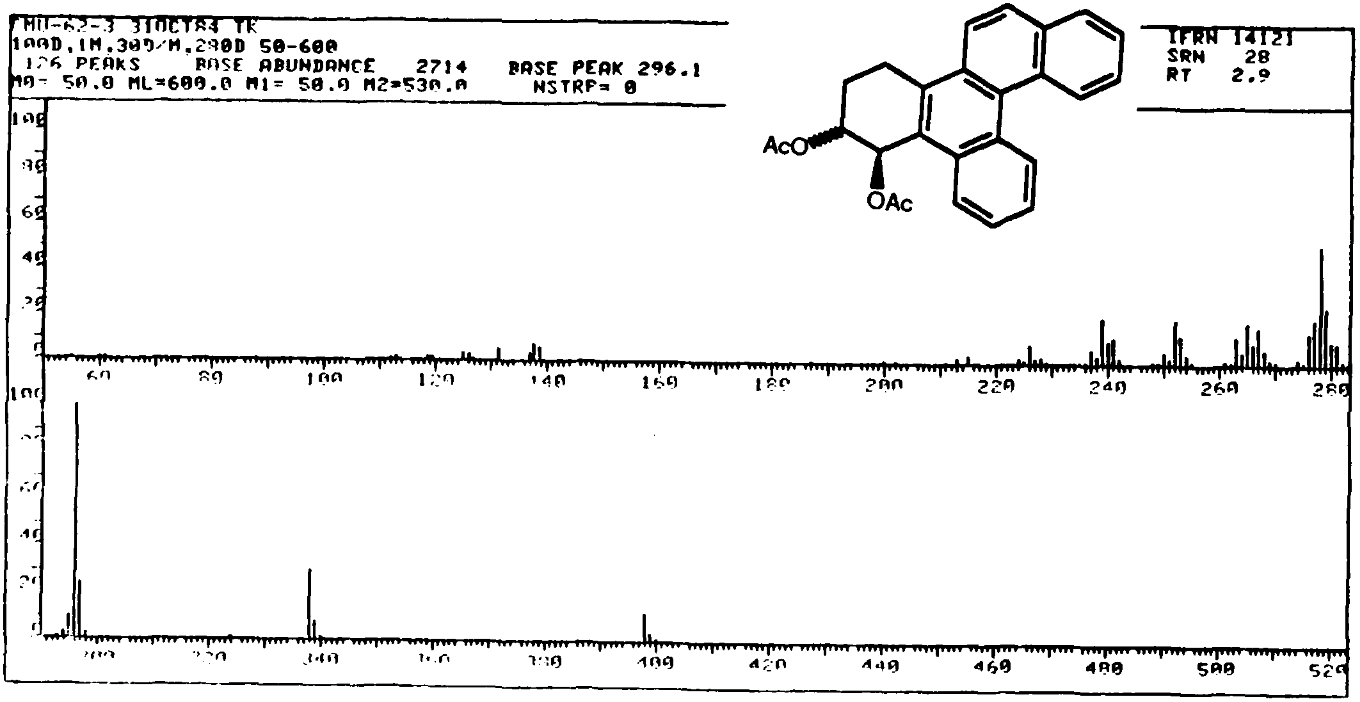
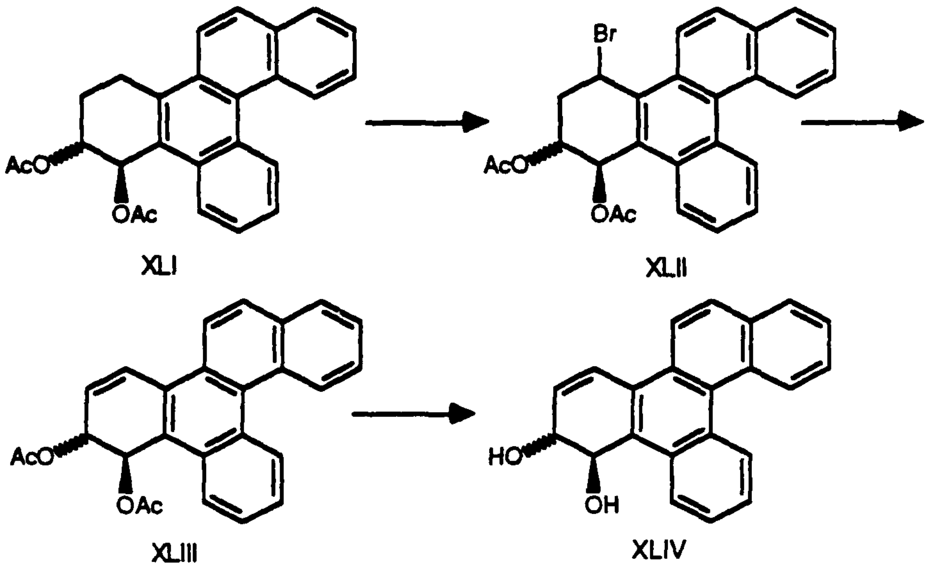


Figure 3.19 70 eV Mass Spectrum of XLI

Scheme 3.11



The final sequence leading to dihydrodiol XLIV exhibited a high degree of sensitivity to reaction conditions and procedures. Earlier, we discovered that bromination of tetrahydrodiacetates was sensitive to any acid (Chapter 2). We assumed this would also hold true for tetrahydro diacetate XLI. We again washed the reaction vessels with dilute ammonium hydroxide before drying. Bromination using N-bromosuccinimide in carbon tetrachloride took place in 2 hours at 50°C. Before the reaction mixture was removed from the heat, the progress of the reaction was checked by NMR. The bromodiacetate wasn't isolated. However, a mass spectrum of the crude material gave a molecular ion at m/e 476 and 478 of equal intensity and a large peak at 396 (71%) for M^+-80 and M^+-82 showing loss of HBr.

Thermal dehydrobromination of XLII was attempted by continuing to heat the reaction mixture at 50-60°C for 72 hours. An NMR spectrum at that time indicated that dehydrobromination had started; however, less than 50% of the material was dihydrodiol diacetate XLIII. In addition, it appeared that further thermal decomposition of the product was occurring.

An alternative method was effective and has been used in earlier preparation of dihydrodiacetates (12). In this case 1,5-diazabicyclo[4.3.0]non-5-ene (DBN) in anhydrous THF gave a 51% conversion to XLIII. However, work up of the reaction provided some concern.

This sequence of the synthesis was sensitive to acids and the standard procedure to remove excess DBN involves washing with dilute hydrochloric acid. To avoid acids we attempted washing the mixture with distilled water several times without acid. However, this apparently did not sufficiently remove DBN, and products ranging from dihydro diol and partially deprotected dihydrodiols to completely aromatized products developed when the solvent was removed.

Fortunately, the dihydrodiol diacetate was able to withstand a cold, dilute acid wash. In this way XLIII was isolated. See Figures 3.20 and 3.21 for NMR and mass spectra.

Deprotection of the dihydrodiol diacetate XLIII was accomplished using methanol saturated with anhydrous ammonia. Complete deprotection occurred upon stirring the

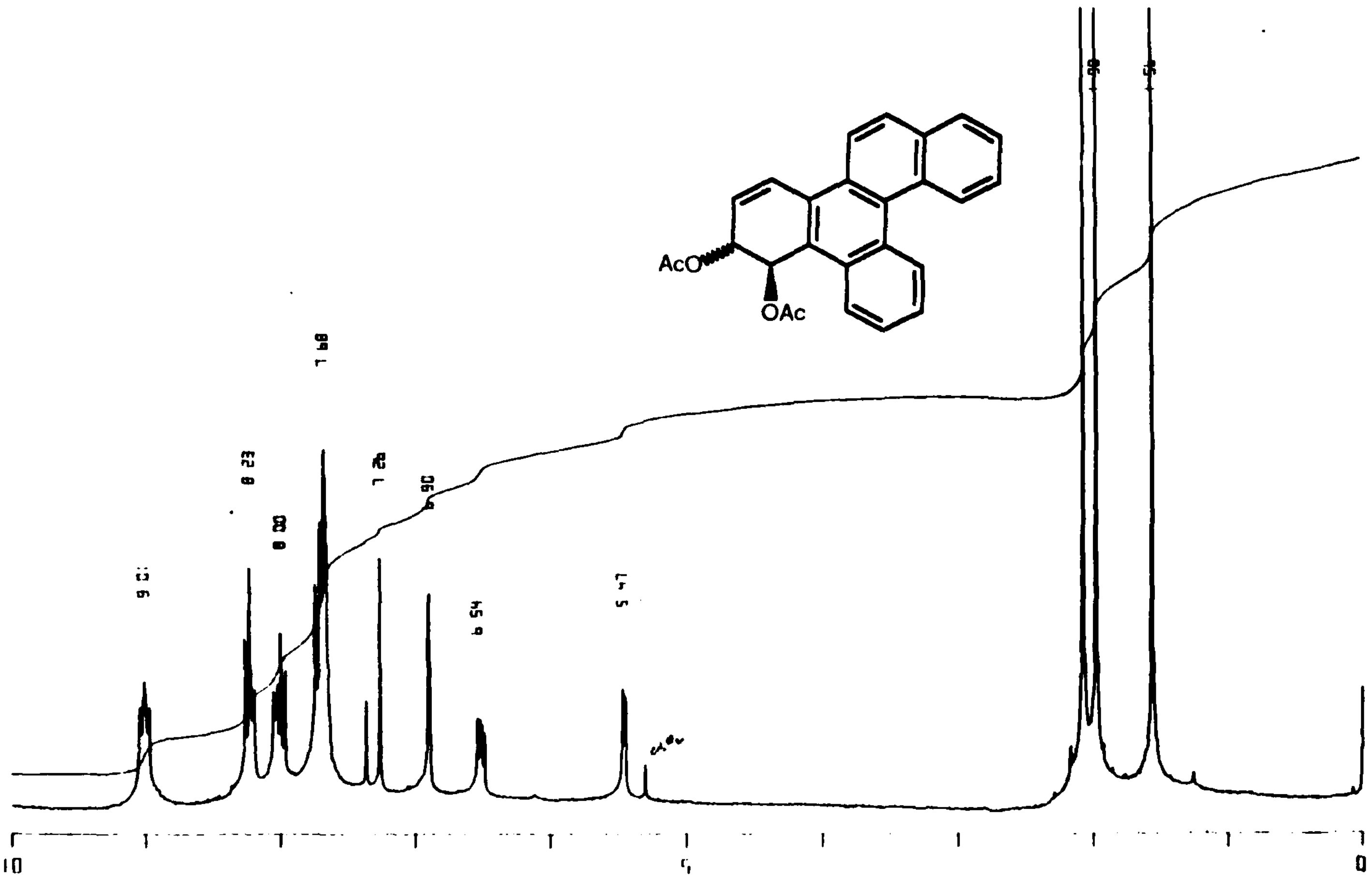


Figure 3.20 300 MHz NMR Spectrum ($CDCl_3$) of XLIII

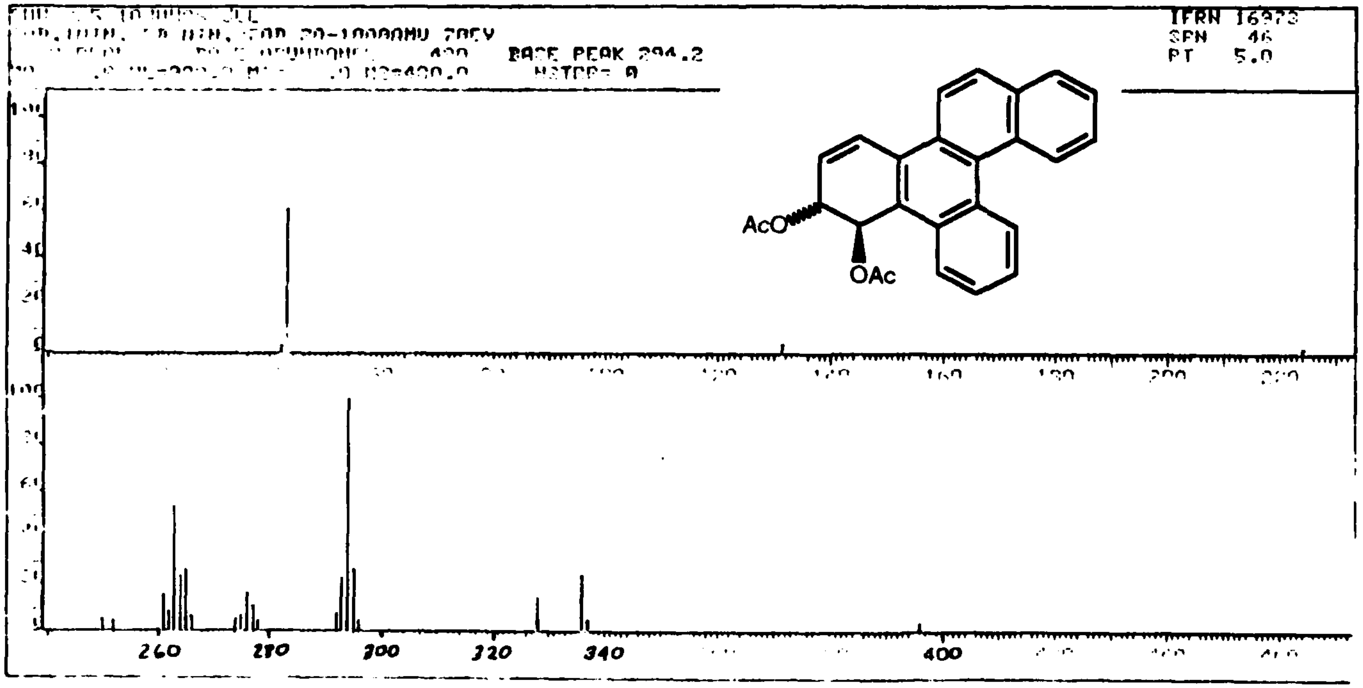


Figure 3.21 70 eV Mass Spectrum of XLIII

reaction mixture overnight at room temperature. Again, the dihydrodiol was found to be sensitive to purification procedures. Initial attempts to use column chromatography caused extensive decomposition. These attempts involved loading crude XLIV on dry column grade silica gel without prior solvent addition. However, by simply packing the column with benzene, decomposition was reduced and we were able to isolate dihydrodiol XLIV in 30% yield from XLI.

Table 3.3

Proton NMR Results for 5,6-dihydro-5,6-diacetoxy and 5,6-dihydro-5,6-dihydroxybenzo(g)chrysene

	H_5	H_6	H_7	H_8	$-OAc_5$	$-OAc_6$
XLIII	6.90	5.47	6.54	7.72	2.07	1.98
	($J_{5,6}$=1.8Hz, $J_{6,7}$=5.4Hz, $J_{7,8}$=9.7Hz)					
XLIV	5.61	4.59	6.60	7.63		1.89($-OH_6$)
	($J_{5,6}$=1.1Hz, $J_{5,OH}$=6.9Hz, $J_{6,7}$=5.7Hz, $J_{6,OH}$=7.2Hz, $J_{7,8}$=9.8Hz)					

The results of proton NMR experiments are shown in Table 3.3 for compounds XLIII and XLIV. Proton assignments were verified by homonuclear decoupling. As was predicted at the beginning of this synthesis, the vicinal dihydroxy conformation is heavily weighted to diaxial. This is confirmed by the small coupling constants between protons 5 and 6 (1.8Hz for diacetoxy and 1.1 Hz for dihydroxy derivatives). The NMR and mass spectra of dihydrodiol XLIV are shown in Figures 3.22 and 3.23.

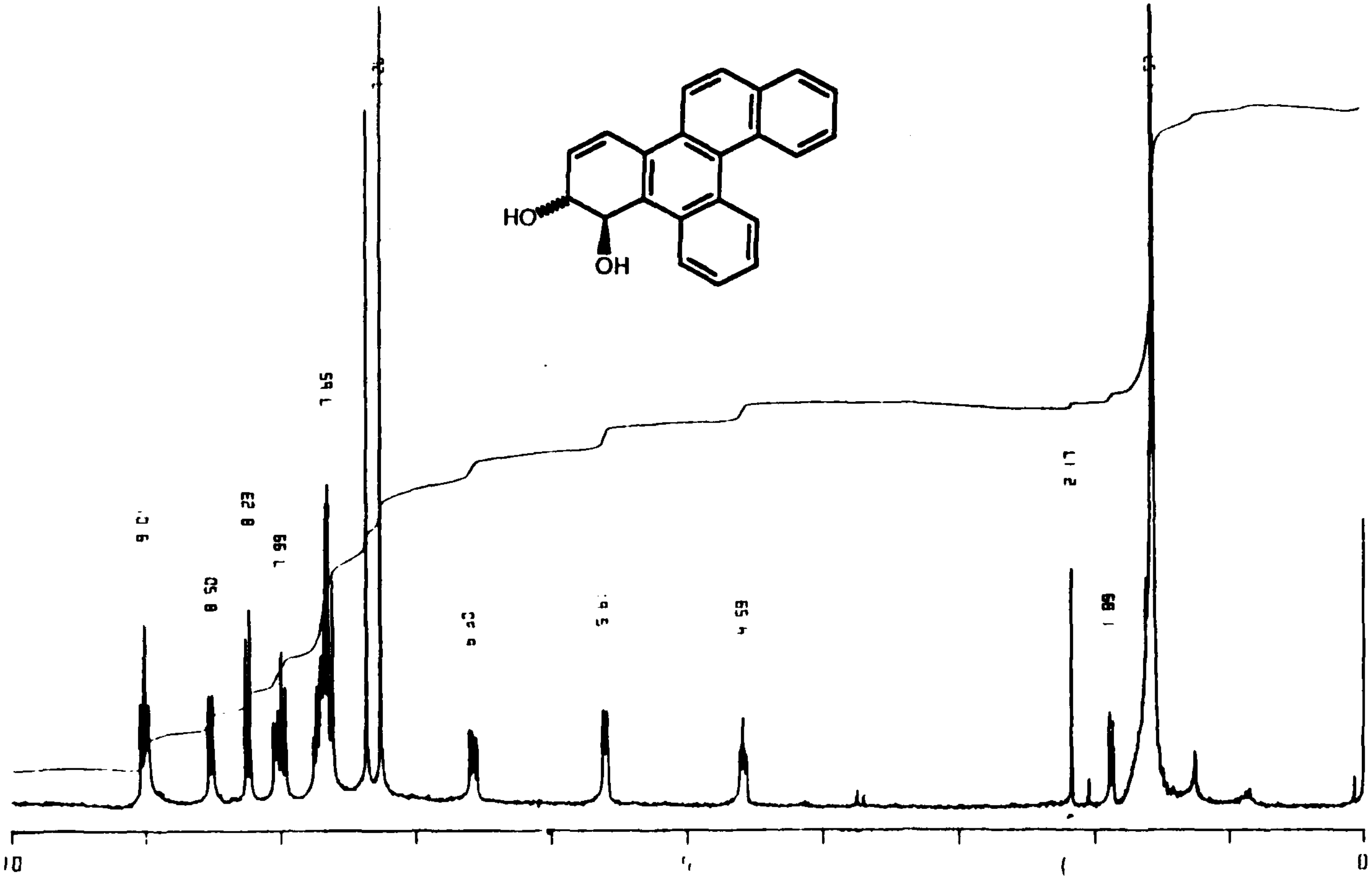


Figure 3.22 300 MHz NMR Spectrum ($CDCl_3$) of XLIV

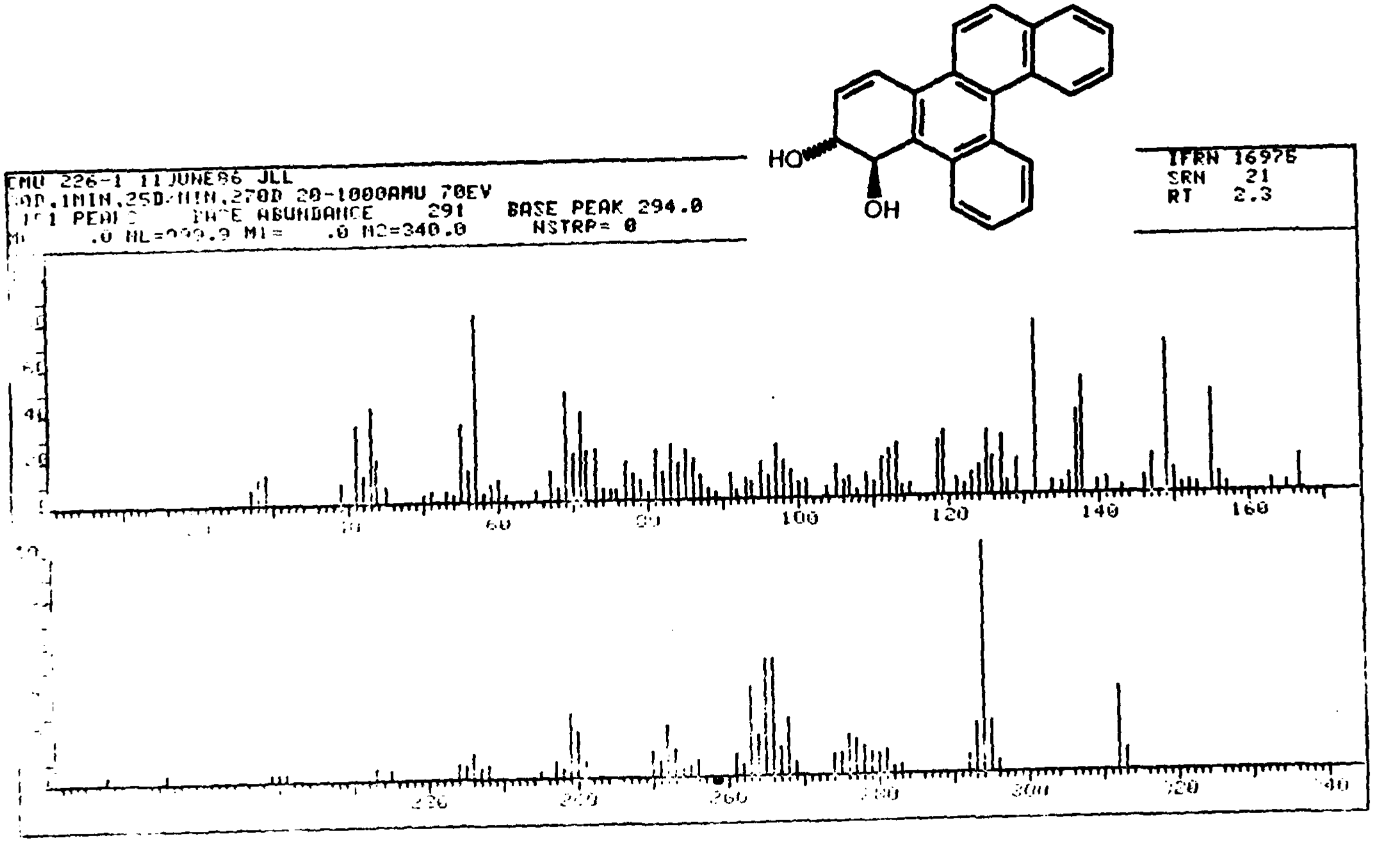
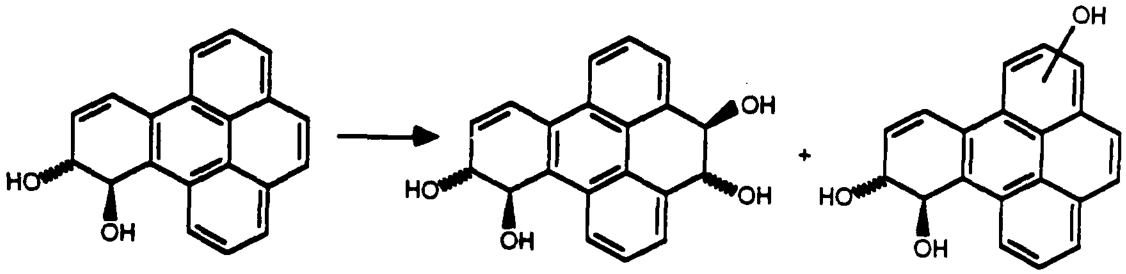


Figure 3.23 70 eV Mass Spectrum of XLIV

A quasi diaxial conformation of the vicinal hydroxyls appears to have a major dampening effect for the overall carcinogenicity of the metabolite. However, biological relationships with this particular molecule are unknown. Benzo(e)pyrene 9,10-dihydrodiol is metabolized at the K-region (Figure 3.24). The lack of formation of bay region diol epoxides has been suggested as one reason for mild or no carcinogenic activity of the 9,10-dihydrodiol. The quasi diaxial hydroxyl arrangement was also credited with directing enzyme activity to distant ends of the molecule (13).

Figure 3.24 (13)

Metabolism of Benzo(e)pyrene 9,10-dihydro diol by Rat Liver Microsomes

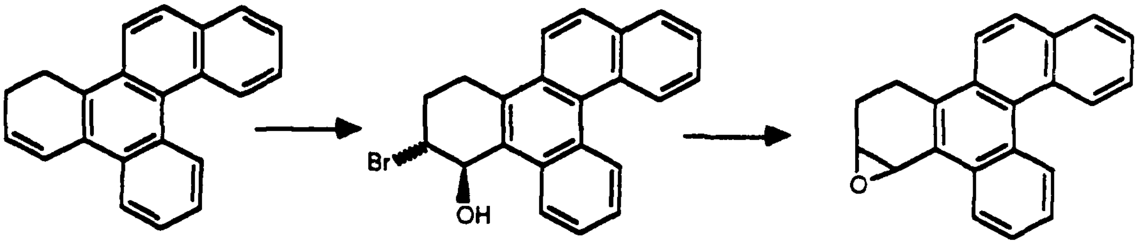


In 5,6-dihydro-5,6-dihydroxybenzo(g)chrysene, XLIV, the same type of outcome may result. However, in this case, additional bay regions are available for metabolism to take place, and may lead to the formation of a carcinogen.

Preliminary studies indicate that XLIV is not a principal metabolite of benzo(g)chrysene when oxidation is effected with cytochrome P450 and rat microsomes (14).

However, that does not rule out metabolism of synthetic XLIV to a biologically active molecule by further metabolism of the 11, 12, 13, 14 benzo ring. Although XLIV doesn't appear to be in the metabolic pathway for activation of benzo(*g*)chrysene, biological testing will determine if it will be mutagenic or tumorigenic in its own right.

Scheme 3.12

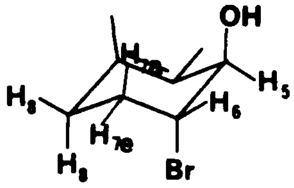


XXXIX XLV XLVI

The second target molecule in this synthetic sequence is tetrahydroepoxide XLVI. These epoxides are prepared by peroxyacid oxidations of dihydro derivatives of polycyclic aromatic hydrocarbons or by bromohydrin/dehydrobromination routes. Because of the success in forming epoxides using polymer supported bases, the bromohydrin route was selected.

Alkene XXXIX was transformed to bromohydrin XLV using 25% aqueous THF and N-bromoacetamide. After the addition of one drop of 6N hydrochloric acid, the bromohydrin rapidly formed and the reaction was essentially complete after 45 minutes. After purification the reaction yielded a white solid, melting point 72-74.5°C. A sample sent for elemental analysis gave satisfactory agreement. In addition, mass spectra provided molecular ions at 378 and 376 (12eV) indicative of monobromination. The base peaks were at 358, 360 (loss of H_2O), (Figure 3.25).

Scheme 3.13



The NMR spectrum of XLV (Figure 3.26) is consistent with the "axial" conformation of the hydroxy and bromine, as shown in Scheme 3.13. The coupling constant between H_5 and H_6 is approximately 3Hz indicating a diequatorial arrangement of the two protons. The multiplet signal at 2.66-2.71 is assigned to H_{7e} based on typical deshielding of an equatorial proton relative to axial of 0.1 to 0.7ppm. Other NMR assignments for the benzo ring are 5.72(dd, J=5.5Hz, 3Hz, H_5), 4.76(m, H_6), 3.40-3.48(m, $2H_8$), 2.66-2.71(m, H_{7e}), and 2.42-2.49(m, H_{7a} + OH).

Conversion of XLV to tetrahydroepoxide XLVI went in 87% yield using Amberlite IRA-400(OH) ion-exchange resin with anhydrous THF as the solvent. The mass spectrum (Figure 3.27) at 12eV gave the expected molecular ion at m/e 296. In addition, high resolution mass spectra confirmed the molecular formula was $C_{22}H_{16}O$. (Calculated m/e is 296.1206, found 296.1220$\pm$.0018). Proton NMR assignments, confirmed by decoupling experiments, for the tetrahydro epoxide ring are (Figure 3.28): 4.83(H_5), 3.96(H_6),

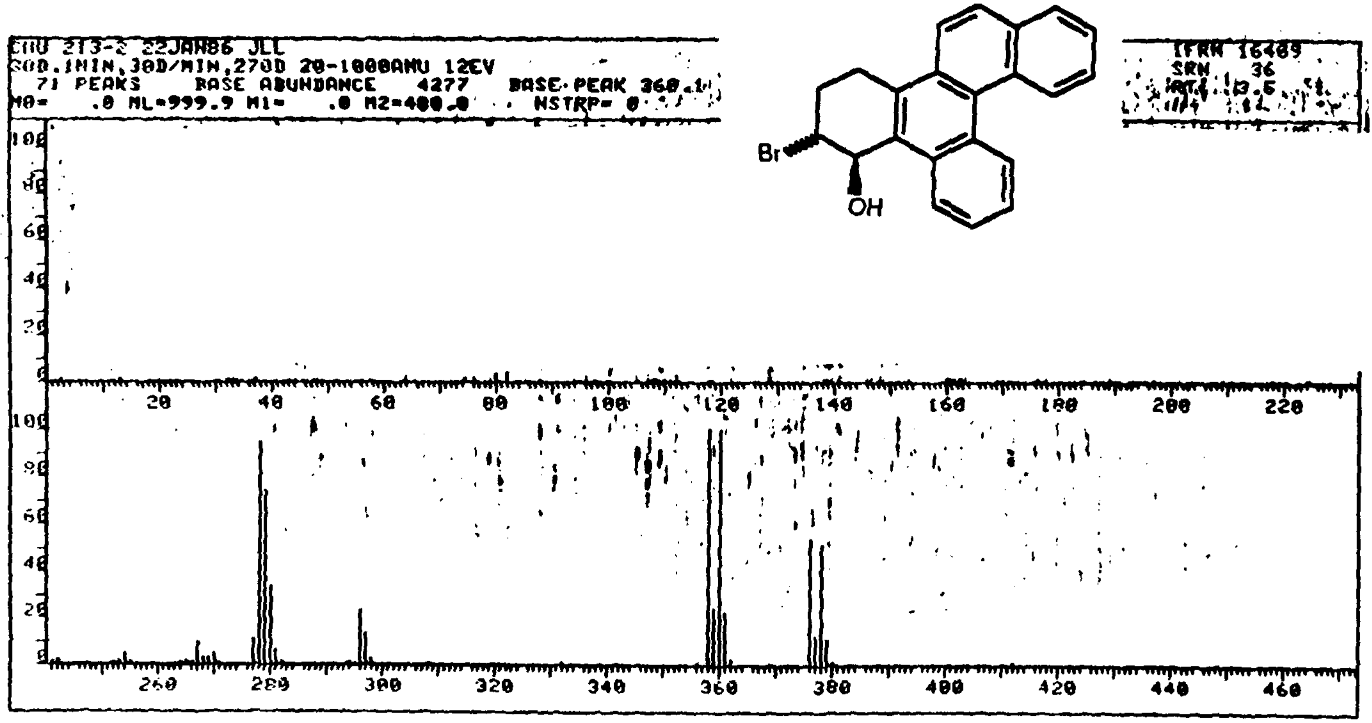


Figure 3.25 12 eV Mass Spectrum of XLV

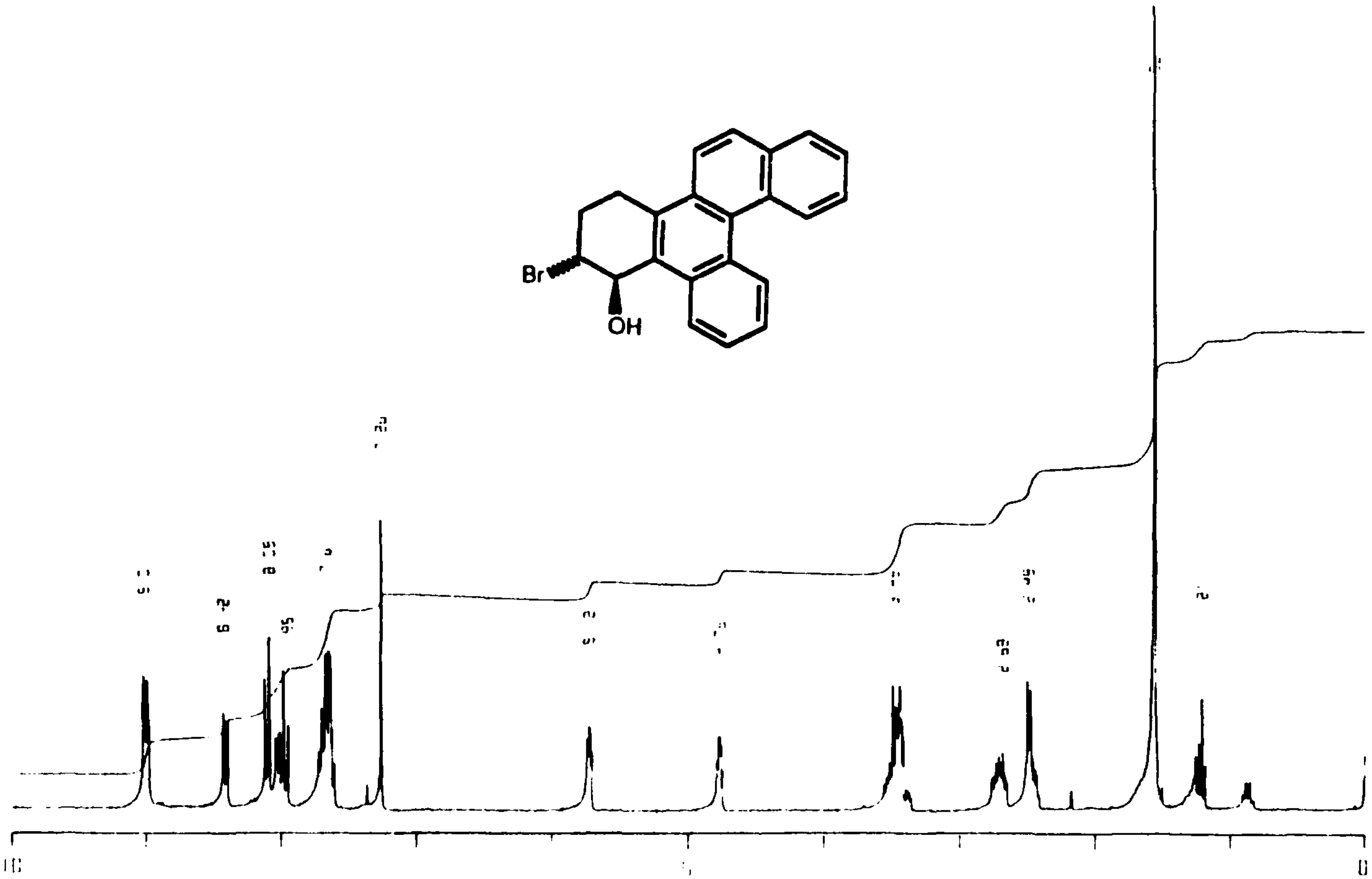


Figure 3.26 300 MHz NMR Spectrum ($CDCl_3$) of XLV

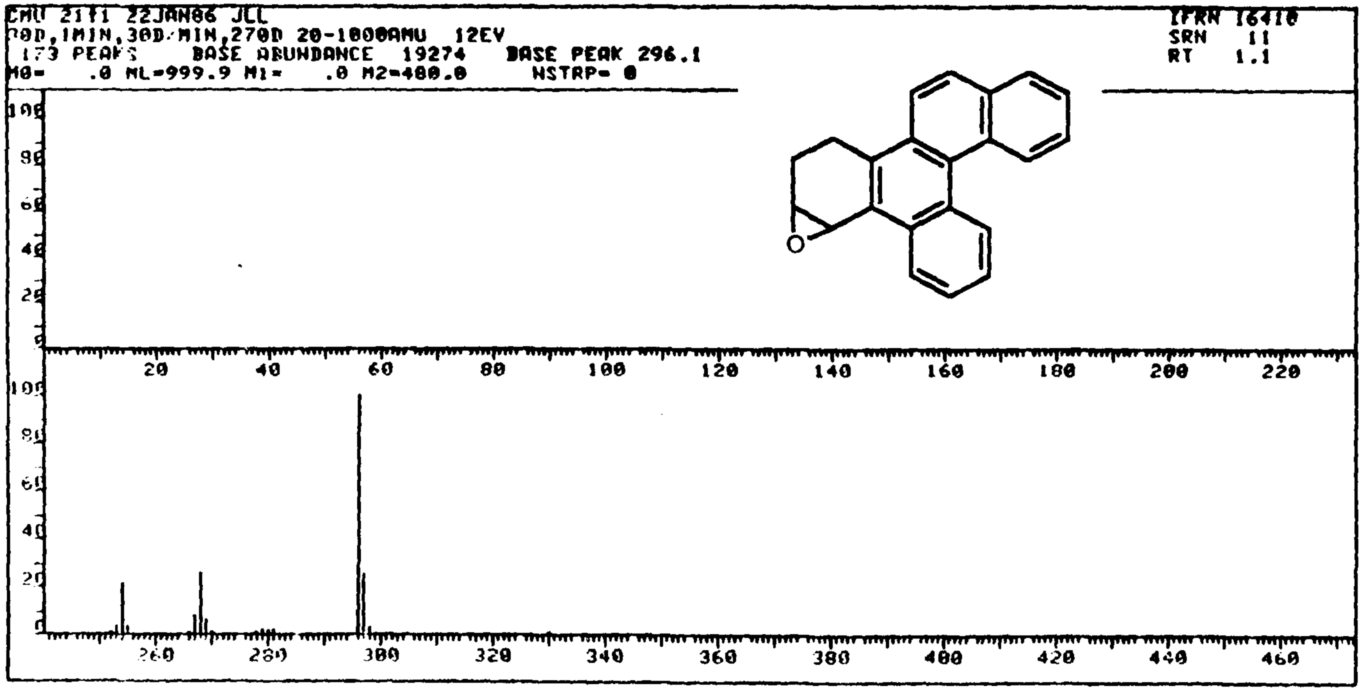


Figure 3.27 12 eV Mass spectrum of XLVI

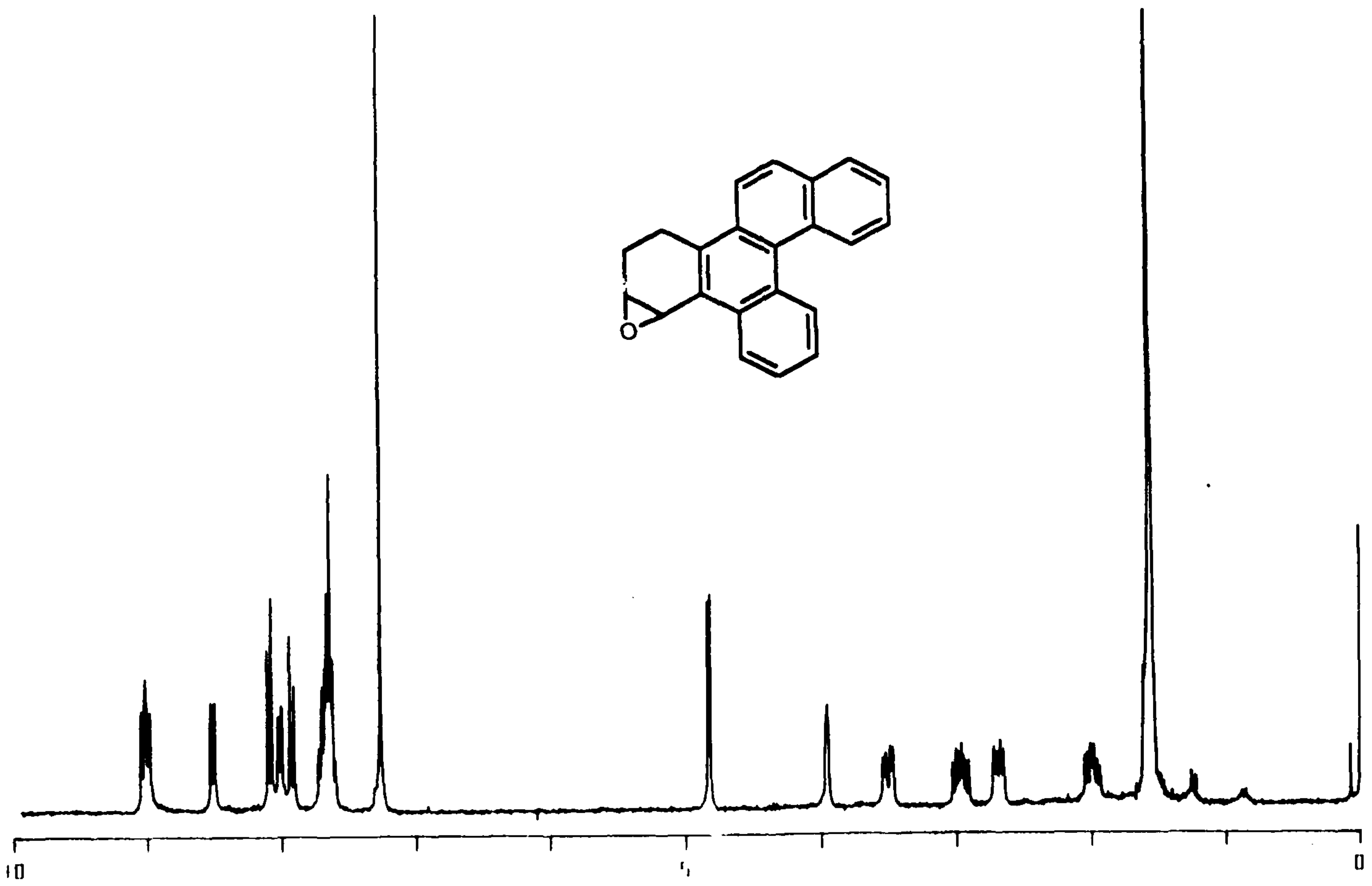


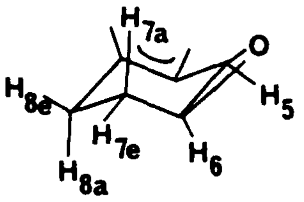
Figure 3.28 300 MHz NMR Spectrum ($CDCl_3$) of XLVI

3.49(H_{8e}), 2.96(H_{8a}), 2.68(H_{7a}), 2.00(H_{7e}). Figure 3.29 gives coupling constant information.

Figure 3.29

5,6-Epoxy-5,6,7,8-Tetrahydrobenzo(g)chrysene

NMR Coupling Constant Data



H	J
5-6	4.5Hz
6-7a/7e	~1Hz
7a-8e	2.0Hz
7a-8a	7.1Hz
7e-8e	6.4Hz
7e-8a	5.0Hz
7a-7e	14.6Hz
8a-8e	16.1Hz

The structure of the tetrahydroepoxide has very interesting characteristics. It is difficult to determine whether the molecule will respond biologically closer to benzo(e)pyrene or chrysene. The calculated value of $\Delta E_{deloc}/\beta$ for the benzylic epoxide position is 0.667 as compared to 0.714 for benzo(e)pyrene and 0.639 for chrysene. Thus far in the correlation studies, mutagenicity increases with increase in $\Delta E_{deloc}/\beta$. Therefore one would predict XLV would be about midway in activity between these two.

Tumorigenicity may increase the interest in this epoxide. There are many structural similarities between chrysene and this benzo(g)chrysene derivative. Since chrysene-3,4-tetrahydroepoxide is tumorigenic, one could anticipate XLV may also be tumorigenic without activation. Biological studies need to be done on this molecule to determine its activity. If these studies find that it does cause tumors, it is likely that 5,6-dihydrobenzo(g)chrysene is a metabolic precursor to the tetrahydroepoxide and both compounds are now available to study such a potential path.

SUMMARY

This chapter described the synthetic sequence used in the preparation of 5,6-dihydro-5,6-dihydroxy benzo(g)chrysene and 5,6,7,8-tetrahydrobenzo(g)chrysene-5,6-epoxide. These compounds were prepared to determine possible metabolites for the carcinogenic PAH benzo(g)chrysene. Initial studies have shown that the 5,6 dihydrodiol is not a major metabolite of the parent hydrocarbon. That is not surprising since quasi diaxial hydroxyl conformations have attenuated biological activity in past studies of PAH metabolites. However, discussion was presented which indicated that the 5,6-dihydrodiol could be metabolized to a more active form, perhaps at another bay region of the molecule.

Tetrahydroepoxides have been traditionally prepared to test the "bay region theory" to avoid complications caused by the vicinal hydroxyls. Correlation between predicted mutagenic activity and $\Delta E_{deloc}/\beta$ has been excellent. In terms of tumorigenicity, only the chrysene bay region tetrahydroepoxide has thus far been found to have activity among PAH without further activation. If the tetrahydroepoxide prepared in this study also demonstrates tumor activity, its likely metabolic precursor, 5,6-dihydrobenzo(g)chrysene, is also available to examine a possible metabolic path toward tumor development.

EXPERIMENTAL

All melting points were obtained on a Kofler melting point apparatus and are uncorrected. Mass spectra were obtained on a Hewlett-Packard 5985 quadrupole mass spectrometer operated at 12 or 70eV. High resolution mass spectra were obtained using a Kratos MS25RF double focusing mass spectrometer with perfluorokerosene as the internal reference. Proton magnetic resonance spectra (300MHz) were obtained on a Varian XL-300 spectrometer. Ultraviolet spectra are from a Perkin-Elmer Lambda 3 spectrophotometer. Preparative photolysis was accomplished using a Rayonet RS Preparative Photochemical photometer at 300nm. NMR values are given in delta values (ppm) downfield from internal standard tetramethylsilane with $CDCl_3$ as the solvent. Cyclohexane was prepared for photolysis reactions by stirring with concentrated sulfuric acid before distillation. Hexane was also treated with concentrated sulfuric acid, washed and then distilled from calcium sulfate. Super dry ethanol refers to absolute ethanol that was refluxed with magnesium metal and a trace of iodine for two hours, then distilled prior to use. Tetrahydrofuran was dried by reflux and distillation from calcium hydride.

Anhydrous benzene was prepared by refluxing and distillation from sodium metal. Microanalyses were performed by Galbraith Laboratories, Inc., Knoxville, Tennessee.

1-(2-naphthyl)cyclopentene (XXXI)

Magnesium metal (1.607g, 0.0661 mole) was crushed and placed in a 3 neck round bottomed flask equipped with mechanical stirrer, reflux condenser with drying tube, and dropping funnel. The apparatus was flame dried and allowed to cool while nitrogen was passed through. 2-Bromonaphthalene (13.857g, 0.0669 mole) in 50ml dry tetrahydrofuran (THF) was added dropwise to the flask after the reaction started. The best way to start the reaction was to add approximately 10% of the alkyl halide in a minimal amount of THF and apply heat via heating mantle to sustain the reflux. After addition of the alkyl halide was complete, stirring was continued for 2 hours at reflux. The mixture was then cooled and cyclopentanone (5.620g, 0.0669 mole) in 30ml THF was added dropwise with stirring while the reaction was controlled via external ice water bath. After addition, the reaction turned reddish brown, but stirring was continued overnight. The magnesium salt was hydrolyzed with water and 6N hydrochloric acid to break up the emulsions that formed. Ethyl ether was added and the aqueous layer was extracted three times with ether (400ml total). The organic layer was washed with saturated salt solution, dried (sodium sulfate) and the ether was evaporated to give benzylic alcohol, 1-(2-naphthyl)cyclopentanol.

The crude alcohol above was transferred to a 250ml round bottomed flask and 100ml benzene and a few crystals of para-toluenesulfonic acid were added. Reflux of this mixture continued for two hours. The reaction was then cooled and the solution was washed with water, saturated salt solution, dried, and the solvent was removed by evaporation. The crude product was purified by flash chromatography using petroleum ether as the solvent. Evaporation of the petroleum ether gave 11.156g XXXI (86%). NMR: 7.69-7.88(m, 4H), 7.42-7.51(m, 3H), 6.34(t, J=2.2Hz, 1H), 2.86(m, 2H), 2.61(m, 2H), 2.09(m, 2H).

4-(2-naphthoyl)butyric acid (XXXII)

A 500ml three necked flask was equipped with an overhead mechanical stirrer. To this flask was added 1.13g (.00582mole) of alkene XXXI, 0.112g of 18-crown-6 ether, and 300ml of benzene. To act similarly to a ball mill, enough glass beads were added to sufficiently grind the reactants, yet not bind the stirrer. Finally, 1.84g (.0116mole) of potassium permanganate was added and the stirring was continued for four hours at room temperature. Solid sodium bisulfite and 6N hydrochloric acid were added alternately until any unreacted permanganate or formed manganese (IV) oxide was destroyed. When the solution became colorless, the two layers were separated. The organic layer was saved and the aqueous layer was extracted with methylene chloride. These extracts were combined with the saved organic layer and extracted with 0.5N sodium hydroxide until the aqueous layer became colorless and the extract was basic (pH paper). The basic extracts were combined and made acidic by dropwise addition of hydrochloric acid and were extracted with methylene chloride. The organic fraction was washed with saturated salt solution, dried, and the solvent was evaporated to give 0.7839g of XXXII (55.7% yield). NMR: 8.49(s, 1H), 7.86-8.05(m, 4H), 7.58(m, 2H), 3.23(t, J=7.1Hz, 2H), 2.56(t, J=7.1Hz, 2H), 2.16(m, 2H).

4-(2-naphthoyl)-methylbutyrate (XXXIII).

Method 1. In a 250ml round bottomed flask equipped with reflux condenser, magnetic stirrer and heating mantle was placed 2.659g of crude keto acid XXXII. Methanol (100ml) and 10 drops of concentrated hydrochloric acid were added. The mixture was refluxed for two hours, the methanol was removed at reduced pressure and the residue was dissolved in methylene chloride. This solution was washed with water, 5% sodium bicarbonate solution, water, dried, and the solvent was removed. The ester could be recrystallized from ethanol/petroleum ether giving 1.9198g (68.2%).

Method 2. This method involves the Friedel-Crafts acylation of naphthalene and is described in detail in chapter two, compound XIII. Of the 18.4348g of crude product in this reaction, 4.012g (12.9% yield), of XXXIII was produced. NMR: 8.48(s, 1H), 7.86-8.05(m, 4H), 7.54-7.63(m, 2H), 3.70(s, 3H), 3.19(t, J=7.2Hz, 2H), 2.49(t, J=7.2Hz, 2H), 2.14(m, 2H). Melting point was 86-88°C.

5-(2-naphthyl)-6-phenyl-ethylhex-5-enate (XXXIV)

Sodium metal (.110g) was added to 15ml of super dry ethanol to prepare sodium ethoxide. Benzyl triphenyl phosphonium chloride (1.8752g) was placed in a 250ml round bottomed flask equipped with magnetic stirrer and dropping funnel. Super dry ethanol (10-15ml) was added to dissolve the salt. Then the sodium ethoxide solution was added dropwise over a 20 minute period. At that time the solution turned light cloudy yellow. Keto ester XXXIII (.701g, 0.00274 mole) was added and rinsed with additional ethanol (the ethanol had to be heated to dissolve the keto ester). The dropping funnel was replaced with a reflux condenser and the mixture was refluxed for 16 hours before going to completion. The ethanol was removed by rotovapor and the residue was dissolved in methylene chloride, washed with water, dried, and the solvent was evaporated. The residue was placed on a column of silica gel and twice purified by flash chromatography using benzene as eluent. After evaporation of the benzene, .8391g (89%) of XXXIV remained as a 56:44 mixture of E:Z isomers. Mass spectrum m/e: 344, 299, 256, 165. NMR: 6.90(s, 1H)-E isomer; 6.56(s, 1H)-Z-isomer.

4-(6-benzo(c)phenanthrenyl)-ethyl butyrate (XXXV)

In a 3 liter pyrex reaction vessel was placed 0.830g of alkene XXXIV, 1 liter of distilled cyclohexane, and 50mg of iodine. The vessel was placed in a photolysis apparatus equipped with an air inlet tube, magnetic stirrer, and condenser. Air was bubbled into the reaction flask after it passed through solid potassium hydroxide pellets and anhydrous calcium sulfate. Photolysis at 300nm was started and continued for 40 hours (until an aliquot showed 90+% reaction by NMR). After that time the solution was yellow in color. The cyclohexane was removed by rotovapor and the residue was quickly filtered through a short column of silica gel with 5% ethyl acetate in benzene. Evaporation of the solvent provided .88g of crude ester XXXV. NMR: downfield multiplet 9.01-9.10(m, 2H) characteristic of H_1 and H_{12} of benzo(g)chrysene.

4-(6-benzo(c)phenanthrenyl) butyric acid (XXXVI)

To a 100ml round bottomed flask equipped with magnetic stirrer and reflux condenser was added 2.51g of the crude ester XXXV, 60ml of water, and 4g of potassium hydroxide. The mixture was brought to a boil and reflux was continued for 1.5 hours. It was then cooled, additional water was added, and the mixture was extracted with ether. The organic layer was discarded and the aqueous layer was acidified with hydrochloric acid, and extracted with methylene chloride. The organic phase was dried and solvent was removed. The residue was crystallized with ether/petroleum ether providing 1.5917g of XXXVI (72.7% from XXXIV), melting point 120-122°C. NMR data: 9.01-9.10(m, 2H), 8.10(d, J=6.9Hz, 1H), 8.02(dd, J=7.6, 1.95Hz, 1H), 7.96(d, J=9Hz, 1H), 7.94(d, J=8.3Hz, 1H), 7.74(s, 1H), 7.57-7.69(m, 4H), 3.27(t, J=7.7Hz, 2H), 2.55(t, J=7.2Hz, 2H), 2.20(m, 2H). Mass spectrum m/e 314, 254, 253, 241.

7,8-dihydrobenzo(g)chrysene-5(6H)-one (XXXVII)

To a 100ml round bottomed flask equipped with magnetic stirrer was added 1.500g (0.00478mole) of acid XXXVI and 30ml of methanesulfonic acid. The flask was capped with a drying tube and placed in an oil bath maintained at 60°C. After 1.5 hours the contents were cooled to room temperature and poured onto 100g of crushed ice with stirring by glass rod. An additional 100ml water was added and the aqueous layer was extracted with methylene chloride. The organic phase was dried and the solvent was removed by evaporation. The yellow oily residue that remained was purified by flash chromatography with benzene as the solvent. Upon removal of the solvent by evaporation and trituration with ethyl ether, 1.2159g (86%) XXXVII resulted as a light cream colored solid, melting point 138-140°C. NMR data: 9.21 (d, J=7.8Hz, H_4), 8.96(m, 1H), 8.86(d, J=7.92Hz, 1H), 8.11(d, J=8.9Hz, 1H), 8.02(m, 1H), 7.96(d, J=8.9Hz, 1H), 7.58-7.67(m, 4H), 3.48(t, J=6.1Hz, $2H_8$), 2.87(t, J=6.8Hz, $2H_6$), 2.35(m, $2H_7$). Mass spectrum m/e 296, 268, 239.

5-hydroxy-5,6,7,8-tetrahydrobenzo(g)chrysene (XXXVIII)

Ketone XXXVII (1.1659g, 0.00394mole) was placed in a 250ml round bottomed flask containing a magnetic stirrer and was dissolved with 10ml THF. Methanol (60ml) was added and 0.837g (0.022mole) of sodium borohydride was added piecemeal to avoid loss of material due to foaming. Some assistance was obtained by external cooling with an ice bath. After addition of sodium borohydride was complete, the flask was capped with a balloon and stirred at room temperature for one hour. Water (20ml) was then added followed by enough dilute hydrochloric acid to make the reaction acidic by pH paper. This was extracted three times with 20ml methylene chloride. The organic phase was dried, and the solvent was removed by evaporation to give a light yellow solid. This was triturated with petroleum ether and dried, to give 1.0658g (91%) of cream white XXXVIII, melting range 158-162°C. NMR data: 8.98(d, J=7.9Hz, 2H), 8.50(d, J=8.0Hz, 1H), 8.09(d, J=8.9Hz), 8.00(dd, J=6.8, 2.17Hz, 1H), 7.93(d, J=8.9Hz, 1H), 7.62(m, 4H), 5.61(bs, 1H), 3.40(m, 1H), 3.12(m, 1H), 2.02-2.33(m, 5H). Mass spectrum m/e 298, 280, 270, 269.

7,8-dihydrobenzo(g)chrysene (XXXIX)

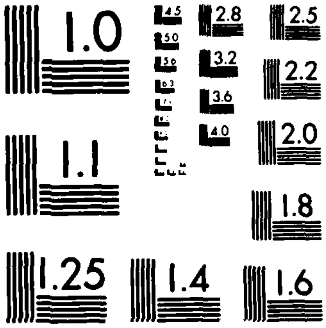
Carbinol XXXVIII, 1.0035g, was dissolved in 100ml benzene in a 250ml round bottomed flask equipped with magnetic stirrer. A few crystals of para-toluenesulfonic acid were added and the flask was capped with a drying tube and placed in an oil bath maintained at 50°C. After 1 hour the dehydration was complete. The benzene solution was cooled, washed with water, dried, and the solvent was removed by evaporation. The residue was purified by flash chromatography using petroleum ether as the solvent. Evaporation gave 0.8550g (91%) XXXIX, a light green oil that wouldn't solidify even at -20°C. UV in methanol: 212(2.6×10^5), 278(141,000), 287(133,000), 299(121,000), 334(32,000), 351(32,000). NMR data: 9.02(m, 2H), 8.30(dd, J=7.0, 2.5Hz, 1H), 8.13(d, J=9.0Hz, 1H), 8.00(dd, J=7.7, 1.8Hz, 1H), 7.92(d, J=8.9Hz, 1H), 7.57-7.90(m, 4H), 7.37(d, J=9.9Hz, 1H), 6.41(m, $J_{5,6}$=9.9Hz, $J_{6,7}$=4.8Hz, 1H), 3.34(t, J=8.3Hz, 2H), 2.54(m, 2H). Mass spectrum m/e 280, 279, 278, 276, 265.

Benzo(g)chrysene (XL)

Method 1. To a 100ml round bottomed flask equipped with magnetic stirrer was placed 258mg (0.921mmole) of alkene XXXIX and 20ml dry benzene. DDQ, 230mg (1.014mmole), was added and the reaction mixture was stirred at room temperature overnight. The following morning the mixture was filtered and rinsed with an additional 10ml benzene. The solvent was concentrated and the residue was purified by preparative thin layer chromatography using petroleum ether as the solvent. Benzo(g)chrysene was removed using methylene chloride and ethyl acetate. The solvent was evaporated leaving 189mg XV (74%), melting point 112-114°C, (lit. 114.5-115°C (15)).

Method 2 In a second procedure, 200mg of 1,2-dihydrobenzo(g)chrysene (.714mmole) and 178mg DDQ (.785mmole) were placed in 20ml dry benzene as in method 1. After purification by flash chromatography, the procedure gave 187mg XL (94% yield). NMR data: 8.90-8.96(m, 2H), 8.60-8.76(m, 4H), 8.00-8.05(m, 2H), 7.57-7.74(m, 6H). Mass

AD-A171 766 SYNTHESIS OF POTENTIAL METABOLITERS IN THE 1234 AND 3/3
5678 BENZO RING POSIT..(U) AIR FORCE INST OF TECH
WRIGHT-PATTERSON AFB OH C M UTERMOEHLEN 1986
UNCLASSIFIED AFIT/CI/NR-86-139D F/G 7/3 NL



MICROCOPY RESOLUTION TEST CHART
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spectrum m/e 278, 277, 276, 274.

trans-5,6-Diacetoxy-5,6,7,8-tetrahydrobenzo(g)chrysene(XLI)

Silver acetate, 0.496g (.00297mole), and iodine, 0.377g (.00148mole) were stirred together in 15ml of dry benzene for 15-30 minutes. When the iodine color had faded, 0.320g (.00114mole), of dihydro PAH XXXIX was added and the mixture was stirred at room temperature. After one hour it appeared by thin layer chromatography that the iodoacetate had formed and the reaction flask was heated to bring the mixture to reflux. After 20 hours the suspended silver salt was vacuum filtered while hot and the filtrate was washed with an additional 15ml of benzene. The solution was concentrated and purified by flash chromatography using first benzene and then 5% ethyl acetate in benzene to elute the diacetate XLI. Evaporation of the solvent gave .3644g of XLI (80%). The white aerosol that resulted was crystallized by dissolving it in ethyl acetate and then adding petroleum ether until cloudiness appeared. Cooling overnight at -20°C gave a white powder melting at 171-175°C. NMR data: 9.01(m, 2H), 8.11(d, J=9.0Hz, 1H), 7.95-8.05(m, 3H), 7.63-7.69(m, 4H), 6.69(d, J=3.3Hz, H_5), 5.41(m, H_6), 3.28-3.43(m, $2H_8$), 2.17-2.44(m, $2H_7$), 2.12(s, 3H), 2.01(s, 3H). Mass spectrum m/e 398, 338, 296.

trans-5,6-diacetoxy-5,6-dihydrobenzo(g)chrysene (XLIII)

Compounds prepared in this sequence are acid sensitive. Before proceeding wash all glassware with dilute ammonium hydroxide and distilled water, then dry the glassware.

To a 50ml round bottomed flask equipped for magnetic stirring was added 119mg (.299mmole) of XLI, 0.50g of solid sodium bicarbonate, 30ml of carbon tetrachloride, and 69mg (.388mmole) of N-bromosuccinimide. The flask was capped with a reflux condenser and drying tube and placed in an oil bath maintained at 55°C. The reaction was maintained at this temperature for 2.5 hours. Then, the solution was transferred to a separatory funnel and washed with water. The organic layer was dried and the solvent was removed by evaporation leaving XLII. The mass spectrum gave m/e 478, 476 indicative of mono bromination. This material was then dissolved in anhydrous THF, 25ml, and 200 drops of 1,5-diazabicyclo[4.3.0] non-5-ene (DBN) was added. The flask was flushed with argon and placed in a freezer at -20°C for 16 hours and then at 0°C for 2 hours. Ethyl acetate, 20ml, was added to the reaction mixture and the organic layer was washed twice with 20ml water, twice with 30ml of 1N hydrochloric acid, and once with 20ml 5% sodium bicarbonate solution, and once again with 20ml water. The organic layer was then dried and the solvent was evaporated. The residue was purified by column chromatography with dry column grade silica gel and 0.7% ethyl acetate in benzene as the solvent. The solvent was evaporated giving 60mg (51%) of white solid dihydrodiacetate (XLIII) melting at 133-136°C. NMR data: 8.97-9.04(m, 2H), 8.24(d, J=9.2Hz, 1H), 8.19-8.22(m, 1H), 8.01-8.07(m, 1H), 7.98(d, J=8.9Hz, 1H), 7.72(d, J=9.7, H_8), 7.60-7.70(m, 4H), 6.90(bs, H_5), 6.54(dd, J=9.7, 5.4Hz, H_7), 5.47(dd, J=5.4Hz, 1.8Hz, H_6), 2.07(s, 3H), 1.98(s, 3H). Analyzed for C,H. Calculated for $C_{26}H_{20}O_4$: C, 78.77; H, 5.09. Found: C, 78.74; H, 5.19. Mass spectrum m/e 396, 338, 294, 263.

<u>trans-5,6-Dihydroxy-5,6-dihydrobenzo(g)chrysene (XLIV)</u>

To a 100ml round bottomed flask was added 17mg of dihydrodioldiacetate XLIII and 10ml methanol. This mixture was cooled to 0°C via ice bath and saturated with anhydrous ammonia. After the ammonia had bubbled through the mixture for 15-20 minutes, the flask was capped with a balloon and the reaction mixture was stirred at room temperature overnight. The next morning the methanol was removed by rotovapor and the residue was dissolved in methylene chloride. The CH_2Cl_2 solution was washed with water, saturated salt solution, and dried. After the solvent was evaporated, the material was purified by column chromatography. The column was prepared by making a slurry with dry grade silica gel and benzene. Dihydrodiol XLIV was eluted with 30% ethyl acetate in benzene giving 11.9mg (39%). This was solidified by adding a small amount of benzene and then petroleum ether. The melting range was 77-80°C. NMR data: 8.97-9.04(m, 2H), 8.50(d, J=8.1Hz, 1H), 8.24(d, J=9.0Hz, 1H), 8.01-8.05(m, 1H), 7.98(d, J=8.9Hz, 1H), 7.66-7.75(m, 4H), 7.63(d, J=9.8Hz, H_8), 6.60(dd, J=9.8, 5.7Hz, H_7), 5.61(dd, J=6.9, 1.1Hz, H_5), 4.59(m, H_6), 1.88(d, J=7.2Hz, $-OH_6$). The second hydroxyl proton is buried under the water peak in the spectrum. Mass spectrum, m/e (rel. intensity) 312 (0.8), 294 (100).

trans-6-bromo-5-hydroxy-5,6,7,8-tetrahydrobenzo(g)chrysene (XLV).

In a 25ml round bottomed flask equipped with magnetic stirrer was placed 67.2mg (.240mmole) of 7,8-dihydrobenzo-(g)chrysene (XXXIX), 9ml freshly distilled tetrahydrofuran and 3ml water. N-Bromoacetamide (36.7mg, 0.264mmole) and 1 drop of 6N hydrochloric acid were added. Stirring was continued for 45 minutes, at which time 10ml ethyl acetate was added to the reaction mixture and the organic layer was washed three times with 10ml portions of water. Thin layer chromatography indicated two products. Separation was by flash chromatography with benzene as the solvent. After the solvent was removed by rotovapor, XLV remained as a white solid, melting point 72-74.5°C. NMR data: 9.00(d, J=6.7Hz, 2H), 8.41(d, J=8.0Hz, 1H), 8.09(d, J=9.0Hz, 1H), 8.02(m, 1H), 7.96(d, J=9.0Hz, 1H), 7.60-7.72(m, 4H), 5.72(dd, J=5.5, 3Hz, H_5), 4.76(m, H_6), 3.40-3.48(m, $2H_8$), 2.66-2.71(m, H_7), 2.42-2.49(m, H_7+OH). Mass spectrum m/e (12eV), 378, 376, 360, 358, 279, 278. Analyzed for C, H, Br. Calculated for $C_{22}H_{17}BrO$: C, 70.04; H, 4.54; Br, 21.18. Found: C, 69.93; H, 4.80; Br, 20.99.

5,6-epoxy-5,6,7,8-tetrahydrobenzo(g)chrysene (XLVI)

To a 10ml round bottomed flask equipped with magnetic stirrer was added 50.8mg, (0.135mmole), of bromohydrin XLV. Amberlite IRA-(400)(OH) ion-exchange resin that had been previously dried using anhydrous THF was added (6ml),along with enough anhydrous THF to barely cover the resin. The reaction mixture was stirred at room temperature for 3 hours. It was then filtered, the Amberlite was washed with an additional 10ml of THF, and the THF was evaporated under reduced pressure yielding 34.8mg (87%) of tetrahydroepoxide XLVI as a white solid, melting range 46-48°C. NMR data: 9.00-9.05(m, 2H), 8.52(d, J=8.7Hz, 1H), 8.09(d, J=8.9Hz, 1H), 8.00-8.03(m, 1H), 7.92(d, J=9.0Hz, 1H), 7.61-7.72(m, 4H), 4.03(d, J=4.5Hz, H_5), 3.96(m, H_6), 3.47-3.55(m, H_3), 2.96-3.00(m, H_8), 2.65-2.72(m, H_7), 1.98-2.05(m, H_7). Mass spectrum m/e 296, 267, 254. High resolution mass spectrum m/e: calculated for $C_{22}H_{16}O$, 296.1206. Found: 296.1220±.0018.

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