THE ISOLATION AND IDENTIFICATION OF SEVERAL CONSTITUENTS OF RAYLESS GOLDENROD, THE REACTIONS OF AZIDES WITH BICYCLO-(2.2.1)-2-HEPTENE AND THE REACTIONS OF BENZENESULFONYL AZIDE WITH AROMATIC COMPOUNDS

By

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CHAPTER I

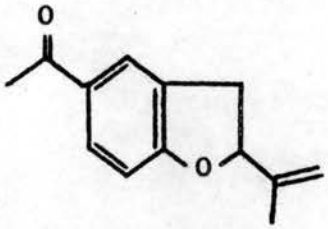
THE ISOLATION OF TOXOL, DEHYDROTREMETONE, AND CARYOPHYLLENE

A. Historical and Introduction

One of the hardships of the American pioneers was a mysterious malady which struck both humans and livestock alike. Known as "trembles" in cattle and "milksickness" in humans, the disease usually appeared in late August and disappeared in late October often leaving scores of deaths behind. Recognized as a distinct disease in North Carolina as early as colonial times, "milksickness" appeared in Ohio, Indiana, Illinois, and other states as the middle west was settled. Later "alkali sickness," a disease clinically identical to milksickness, made its appearance in the southwestern states of Arizona, Texas, and New Mexico. In his Lincoln biography, Carl Sandburg describes this disease which caused the death of Lincoln's mother. "...the 'milksick,' beginning with a whitish coat on the tongue, resulting, it was supposed, from cows eating white snakeroot or other growths that poisoned their milk. ...there came to Nancy Hanks Lincoln that white coating of the tongue; her vitals burned; the tongue turned brownish, her feet and hands grew cold and colder, her pulse slower and slower. Death came October 5, 1818...."[1] The large number of deaths caused by milksickness in the nineteenth century was due in part to the fact that the etiology of the disease was not known. Although it was early suspected that poisonous

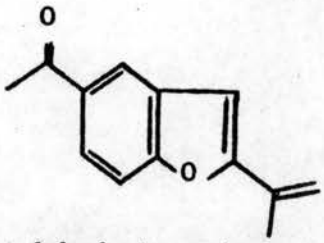
plants might be responsible, the disease was confined mainly to the backwoods and sparsely settled regions and was never prevalent in larger towns where it could come under scientific observation. In the 1920's white snakeroot, Eupatorium urticaefolium Reichard, in the central states and rayless goldenrod, Aplopappus heterophyllus Blake, in the southwestern region of this country were shown to be responsible for the disease.[2,3] Couch was able to isolate a toxic oil considered to be identical from both plants to which he gave the name tremetol.[2,4,5] Couch considered tremetol to be an unsaturated alcohol ($C_{16}H_{32}O_3$, $[\alpha]_D$ - 33.82) of unknown structure; but further work by Dermer[6,7] and his students at this institution showed that tremetol from rayless goldenrod was not a pure compound as had been reported, but rather a complex mixture. However these workers were unable to isolate the pure toxin with the methods available at that time. Apparently no further work was done on these noxious plants during the next twenty years. Fortunately "milksickness" became less of a problem during this period; farmers were educated to recognize and extirpate the poisonous plants from their grazing lands and milk went into large dairy pools where toxin-containing samples became diluted.

Recently, Bonner[8] and co-workers reported the results of their reinvestigation of white snakeroot. By the use of modern chromatographic techniques, these workers found that "white snakeroot tremetol" could be separated into a number of components. Although Bonner isolated three closely related ketones, tremetone, I, dehydrotremetone, II, and hydroxytremetone, III which were toxic to goldfish, none have been proven to be the active toxin in the plant.



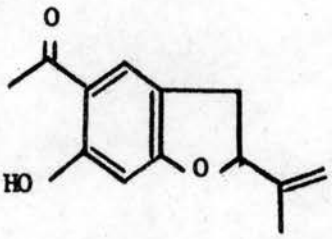
tremetone

I



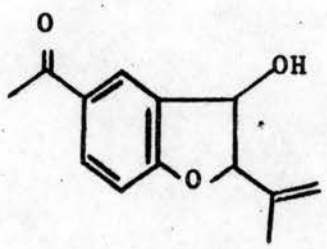
dehydrotremetone

II



hydroxytremetone

III



toxol

IV

B. Results and Discussion

Isolation of Toxol and Dehydrotremetone.

In June of 1961 the reinvestigation of rayless goldenrod was begun and "rayless goldenrod tremetol" was isolated by a procedure similar to that reported by earlier workers.[4,6,7] The first objective was to find a convenient and rapid method of assaying the toxic compound(s) present in the crude toxin (the "rayless goldenrod tremetol" of Couch). Since the many fractions of a chromatographic separation were to be examined, a bacteriological test seemed appropriate. After a rather detailed study, which is described elsewhere[9], Bacillus cereus was

selected as the test organism, and all toxicities reported here refer to inhibition of growth of this organism using the solid agar assay method. The objective was to determine whether the toxic constituents in the rayless goldenrod were identical to those from white snakeroot. In order to do this we attempted to use the partition chromatographic separation on Celite used by Bonner and co-workers.[10] Our crude toxin was found to be only partially soluble (65%) in the mobile phase (ligroin). The ligroin-soluble fraction was found to be toxic whereas the insoluble fraction was non-toxic. Chromatography on Celite according to the procedure of Bonner and DeGraw[10] gave a rapidly eluted toxic fraction which after purification gave a white solid which was found to be identical in all respects to the previously reported dehydrotremetone. A second more polar fraction was eluted only very slowly from the column. When the ligroin-soluble oil was chromatographed on alumina (adsorption chromatography) two distinct toxic fractions were obtained. The less polar fraction was identified as dehydrotremetone as before, whereas the more polar toxic component, toxol, obtained as a viscous dark yellow oil, appeared to be a new compound. Toxol could be obtained as a pure substance only after repeated chromatography on alumina with subsequent preparative thin layer chromatography on silica gel. However, a more efficient and rapid method for obtaining pure toxol using partition chromatography on Florisil was found. The structure elucidation of toxol, IV, has been reported.[9]

Isolation and Identification of Caryophyllene.

Chromatography of "tremetol" on Florisil gave rapidly eluted fractions which were shown to consist almost entirely of hydrocarbons

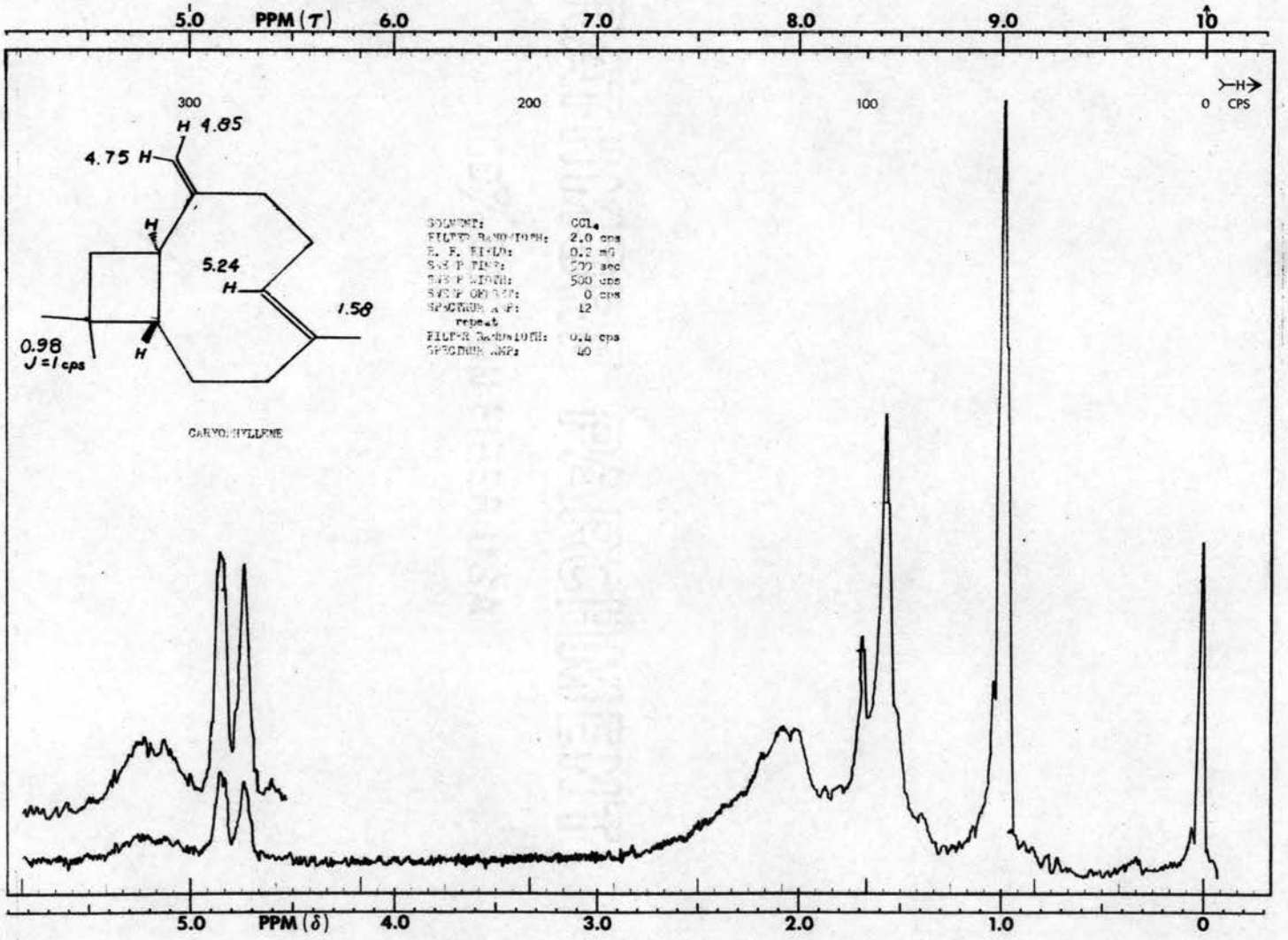
by their infrared spectra. Vapor phase chromatographic (V. P. C.) analysis of these fractions showed that only partial separation of the individual components had been accomplished and that one component accounted for approximately fifty-seven per cent of the total. The fractions were combined and the mixture was fractionally distilled under a nitrogen atmosphere to yield the major component (b.p. $75^{\circ}/0.08$ mm., n_D^{25} 1.4967, $[\alpha]_D$-12.7), which was shown to be over 95% pure by V. P. C. comparison of the compound's infrared spectrum with that given in the literature[11] and N.M.R. analysis (Plate I) indicated the compound was caryophyllene. V. P. C. comparison with an authentic sample positively identified the compound.

C. Experimental

Isolation of "Tremetol" from the Plant.

Rayless goldenrod (Aplopappus heterophyllus Blake) was collected while in full bloom (August, 1960, 1961) just east of Roswell, New Mexico on U. S. Highway 70. The plant was air-dried and just prior to each extraction the aerial organs of the plant were ground in a Wiley mill with a 20-mesh screen. The ground meal was continuously extracted with methanol in a large Soxhlet extractor. In a typical run 2.5 kg. of meal was extracted for 75 hr. with approximately 12 l. of methanol. After standing at 4° overnight the methanolic extract was filtered through glass wool to remove the precipitated plant waxes. The filtrate was concentrated on a steam bath with a water aspirator to give approximately 0.5 l. of viscous, dark green residue. This residue was washed several times with water; the washed residue was dissolved in hot 50% aqueous ethanol (1.5 l.) and the solution filtered while hot. Potassium

Plate 1. Nuclear Magnetic Resonance Spectrum of Caryophyllene.



hydroxide (105 g,) and ethanol were added to the filtrate and the solution was refluxed for 8 hr. Later it was found that the above procedure, which is essentially the same as that used by earlier workers,[4,6,7] could be simplified in the following manner and still yield the same results. The entire supply of the dried aerial organs of the plant was ground in a large mill and stored in heavy paper bags at room temperature until extracted. The plant was extracted and concentrated as above. The residue was taken up in methanolic potassium hydroxide to give approximately 1 l. of a 5% potassium hydroxide solution and then refluxed as above. The alkaline solution after cooling was filtered and concentrated under reduced pressure to a dark gum which was partitioned between water (1 l.) and ethyl ether (3 l.). The ether extract was dried over sodium sulfate and concentrated under reduced pressure to give 15 g. of a viscous red oil, identical to the "tremetol" of earlier workers.

Isolation of Dehydrotremetone; Partition Chromatography on Celite.[9]

Celite (Johns-Manville) was purified by washing with concentrated hydrochloric acid and 95% methanol. Dry purified Celite (20 g) was intimately mixed in a large mortar with 200 ml. of 95% methanol saturated with ligroin, the stationary phase. An excess of ligroin saturated with 95% methanol, the mobile phase, was added and the mixture stirred vigorously. The slurry was poured in portions into a chromatography column, 4 x 35 cm., and allowed to settle by gravity. The mobile phase was added to 8.5 g. of "tremetol" and the soluble fraction (5.4 g.) of "tremetol" was loaded on the column. The column was eluted with the mobile phase and 50-ml. fractions collected. The first

350 ml. of eluent removed 2.0 g. of non-toxic material from the column. The next 450 ml. of eluent gave 2.2 g. of a mixture of viscous orange oil and a white solid. The solid, which proved to be toxic, was found to be identical in melting point and infrared and ultraviolet spectra with dehydrotremetone.[10] The next liter of eluent removed only a negligible amount of material from the column but toxicity tests showed that a new toxic component was being eluted slowly from the column. Total recovery was 4.5 g. (82%).

Adsorption Chromatography on Alumina; Isolation of Toxol.

The mobile phase ligroin-soluble fraction of "tremetol", (30 g.) was chromatographed of alumina (508 g., Merck acid-washed) collecting 100-ml. fractions. Benzene (1500 ml.) eluted 5.4 g. of a viscous orange oil containing a white solid. The solid was found to be toxic and was identified as dehydrotremetone. Another 8.5 g. of non-toxic oils and solids was eluted with benzene-ether and ether (total 2 l.). A second toxic fraction (4.2 g. of viscous dark orange oil) was eluted with 2-6% methanol in ether (2 l.). Higher concentrations of methanol eluted only 1.1 g. of non-toxic material from the column. Methanol-acetic acid failed to elute more material from the column. Total recovery was 19 g. (63%). Gas chromatography of the second toxic fraction using a 0.3-cm. x 1.67-m., 5% SE-30 on Chromosorb W column at 175° showed three major peaks and several minor peaks. Rechromatography of the second toxic fraction on alumina removed the yellow color and gave a viscous colorless oil as the toxic component. However, gas chromatography of the colorless oil with the same column at 190° still showed two major components (RT 0.75, 0.70 min.). Thin layer chromatography on

silica gel G (250 μ, Merck-Darmstadt) using chloroform-methanol (95:5) and spraying with methanolic 2,4-dinitrophenylhydrazine solution showed one major spot (RF 0.75) and several smaller spots. Similar results were obtained by spraying with 5% nitric acid in sulfuric acid. Thin layer chromatography of the other fractions obtained from the alumina chromatography of the second toxic fraction gave chromatograms similar to that of the colorless oil, showing that only partial separation had been achieved. The pure toxic compound, toxol, could be obtained as a colorless oil by repeated preparative thin layer chromatography. However a more efficient and rapid means of obtaining toxol was found by other workers.[9]

Isolation of Caryophyllene and Sterol A.

Florisil (230 g., 60/100, Floridin Co.) was poured into a chromatography column containing the stationary phase (95% methanol saturated with ligroin). After standing overnight, the excess stationary phase was removed and the column was washed with the mobile phase (ligroin) until only the mobile phase was present in the eluent. "Tremetol" (205 g.) was chromatographed on the same column in four runs. The "tremetol" oil was loaded directly onto the column with pressure and eluted with mobile phase (1.5 l.). The column was then stripped with the stationary phase (1 l.) and regenerated for the next run by washing with mobile phase. The appropriate fractions of each run were combined to form four main fractions on the basis of their I. R. spectra and vapor phase chromatography, V. P. C. Recovery was 190 g. (93%).

TABLE I

FLORISIL CHROMATOGRAPHY OF "TREMETOL" OIL

Fraction	ml. per run	combined wt.	wt. of hydrocarbons in fraction	wt. sterol A in fraction
1	500	38.5 g.	8.2 g.	4.6 g.
2	250	33.0	6.4	1.4
3	750	38.3	-	-
4 (strip)	1000	80.0	-	-
		189.8	14.6	5.0

The strip fraction, which contained toxol[9], was set aside. The three remaining fractions were chromatographed individually on alumina (400 g., Merck-basic). Fractions 1 and 2 gave hydrocarbons (eluted with ligroin), and crude sterol (eluted with chloroform) plus unidentified oils. In addition fraction 2 also gave a small amount of dehydrotremetone (0.7 g., eluted with benzene). Fraction 3 gave no hydrocarbons or sterol and its I.R. spectrum indicated that a high percentage of dehydrotremetone and toxol was present. Total hydrocarbons isolated from tremetol was 14.6 g. (7.3%). Total sterol A isolated from tremetol was 5 g. (2.5%). Vapor phase chromatographic analysis of the hydrocarbons showed one major component accounting for 57% of the total. A somewhat cleaner separation of the major component was achieved when the non-ketonic fraction of tremetol (obtained by treating tremetol with Girard's T-reagent[12]) was chromatographed as above. The "cleaner" fractions were combined and the mixture fractionally distilled under a nitrogen atmosphere to yield the major component (b.p. 75/0.08 mm., n_D 1.4967, $[\alpha]_D$ - 12.7) which was shown to be over 95% pure by V. P. C. Anal.

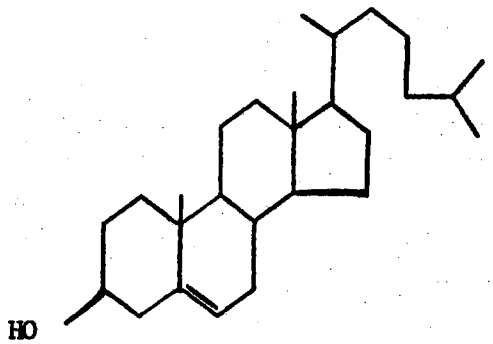
Calcd. for $C_{15}H_{24}$: C, 88.16; H, 11.84%. Found: C, 87.55; H, 11.65%. Comparison of thick film I.R. with that given in the literature[11] and vapor phase chromatography with an authentic sample identified the compound as caryophyllene. V. P. C. data: The experiments were carried out with an Aerograph A-350-B gas chromatograph using helium as the carrier gas, a thermal conductivity cell as the detector, and a Minneapolis-Honeywell strip chart recorder with a 1-mv. scale and a chart speed of 0.5"/min. A ¼", 10' 10% Craig polyester succinate on Chromosorb W column was used. A combined injection of the unknown and an authentic sample of caryophyllene at 160° gave one peak (RT 6.5 min).

CHAPTER II

THE ISOLATION AND PROOF OF STRUCTURE OF STEROL A.

A. Historical and Introduction

A variety of crystalline C_{27} - C_{29} secondary alcohols having high melting points occur in plants and animals. These alcohols have the generic name sterol derived from the Greek stereous, solid. Probably the most familiar individual of the group is the predominant constituent of human gall stones, cholesterol, V, whose structure was elucidated only after years of study by many workers.[13] The sterols found in plants and lower organisms are monohydroxy compounds like cholesterol, but usually possess an additional methyl or ethyl group in the side chain and contain one, two, or three double bonds.[13] Even after the fundamental carbon skeleton of the sterols had been determined, workers had difficulty establishing the presence of methyl or ethyl groups in the side chain. Good chemical analyses of such high molecular weight compounds were (and still are) difficult to obtain and the difference in the carbon-hydrogen ratio between ethyl or methyl groups in the side chain is small. The determination of the position of annular double bonds in sterols is a difficult problem that has caused much confusion in the literature. It was often impossible to decide between the 7:8, 8:9, 8:14, and 9:11 positions by chemical means owing to the facile rearrangements that occurred during reactions.



V

In the 1940's D.H.R. Barton[15-19] extensively studied the optical rotations of all reported sterols and their derivatives. Barton was able to correlate values for the differences between the molecular rotation of sterols and their derivatives with the position of unsaturation in the molecule. Using this method Barton was able to correctly revise the structures reported for several sterols. The greatest limitation of Barton's method of molecular rotation differences (M.R.D.) is that impurities can cause gross anomalies in M.R.D. values. While investigating steroids of unnatural configuration Castells[20] and co-workers developed a new method for locating double bonds in steroids. The method involves cleaving the double bond to a dicarbonyl system, thus generating two groups (aldehyde or ketone) with easily recognized and reliable I.R. bands. This is the method used in determining the position of the annular double bond in sterol A and will be discussed in more detail later. Of all the sterols previously isolated from plants and lower organisms only those with the annular double bond at 5:6, 7:8, and 8:9 positions have been conclusively shown to occur. However no naturally occurring doubly unsaturated sterol has been shown to contain an 8:14 double bond. Examples of mono-unsaturated

sterols with 8:14 double bonds are known since double bonds in 7:8 and 8:9 positions rearrange to the 8:14 position during hydrogenation of the side chain.

B. Results and Discussion

Isolation and Preliminary Studies of Sterol A.

Sterol A was obtained from both partition and adsorption chromatography of "tremetol" as a yellowish amorphous solid which could be crystallized as white needles (m.p. 152-4°, acetone-methanol, $[\alpha]_D^{25°}$ -9°, chloroform). The infrared spectrum of the sterol possessed a band at 970 $cm.^{-1}$ characteristic of trans-disubstituted double bonds in steroid side chains. The low negative rotation of the free sterol indicated that it was not a triterpene or a methyl steroid. The sterol gave a positive Tortelli-Jaffe[21] test, which is specific to steroids containing a tetra-substituted double bond in the ring system or a steroid that is easily isomerized to such a compound. Thus the 7, 8(9), 8(14), and 9(11)-compounds give positive tests. However, the n.m.r. spectrum of the sterol showed only two vinylic protons at δ5.06 corresponding to the vinyl protons in the side chain double bond. This eliminated the 7 - and 9(11) - double bonds as possibilities since either position would have a third vinyl proton in the spectrum. In addition, the n.m.r. spectrum showed a high-field methyl group at δ0.53 similar to the C_{18} methyl group in ergosterol. Indeed the spectrum of dihydroergosterol was nearly identical to that of sterol A which led to the incorrect supposition that sterol A might be impure dihydroergosterol.

The Structure of Sterol A.

Nature of the Side Chain.

Ozonolysis of the sterol and destruction of the ozonide with zinc and water followed by steam distillation of the products yielded a volatile carbonyl compound from which no satisfactory 2,4-dinitrophenylhydrazone or semicarbazone derivatives could be prepared. Tollens oxidation of the volatile compound gave an acid from which the amide was prepared. The unknown amide was compared with 2,3-dimethylbutyramide obtained in a similar manner from ergosterol and 2-ethyl-3-methylbutyramide obtained from ethylisopropylmalonic acid in three steps. The mixed melting points of these compounds (Table IV) indicated that the unknown amide was probably the 2-ethyl compound, but impurities which could not be removed from the amides obtained from the sterols widened the melting range sufficiently to leave some doubt. The three amides were therefore compared by thin layer chromatography which also indicated that the unknown was the 2-ethyl compound. Although the evidence was in favor of a 24-ethyl group in the side chain of the sterol (corresponding to a stigmasterol type structure) rather than a 24-methyl group (corresponding to an ergosterol type structure), it was felt that proof of the entire carbon skeleton was desirable.

The Nature of the Carbon Skeleton.

We felt that the best method for identification of the sterol skeleton would be the V. P. C. comparison of the fully saturated steroid hydrocarbon with cholestane, ergostane, and stigmastane. The major difficulty was the hydrogenation of the annular double bond. Hydrogenation

of the sterol in the presence of platinum catalyst resulted only in the saturation of the side chain double bond as shown by the disappearance of the 970 $cm.^{-1}$ in the I.R., and the absence of the two vinylic protons in the n.m.r. spectra of the dihydro compound whereas the compound still gave positive Tortelli-Jaffe and tetranitromethane tests.

It is known that some steroids can be isomerized with dry hydrogen chloride to an equilibrium mixture of the 8:14 and 14:15 compounds.[18] We felt that hydrogenation of such a mixture would result in the saturation of the 14:15 isomer which then might be easily separated from its unsaturated relative, or the crude hydrogenation mixture could be treated with hydrogen chloride again and rehydrogenated to eventually give the saturated stanol. Both methods were attempted with dihydroergosterol as a model. In our hands the crude hydrogenation mixture could not be separated by alumina chromatography and treatment of the mixture with sulfuric acid resulted in viscous tars. Repeated treatment of the mixture with hydrogen chloride and hydrogenation gave only a greenish viscous oil that gave a positive tetranitromethane test. At this point it was felt that if the isomerization agent could be incorporated in the actual hydrogenation process, the saturation of the sterol might be accomplished. Accordingly dihydroergosterol was hydrogenated in the presence of platinum and perchloric acid. This procedure gave the fully saturated ergostanol in over 90% yield.* Dihydro sterol A was hydrogenated in a similar manner to give its fully saturated analog, Stanol A. Chromic anhydride oxidation of stanol A followed by Wolff-Kishner reduction of the ketone gave the corresponding hydrocarbon.

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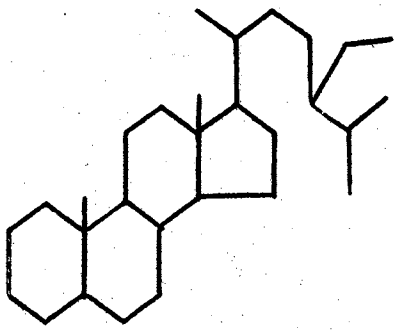
*Later, it was found that Jones, et al. reported the use of perchloric acid with similar results in a recent paper.[22]

Cholestane, ergostane, and stigmastane were prepared in a similar manner from commercial samples of the sterols. After some experimentation, two V.P.C. columns were found that could satisfactorily separate the three known steroid hydrocarbons. Comparison of the unknown with the knowns on both columns showed the hydrocarbon obtained from sterol A to be identical to stigmastane, VI.



VI

The Position of the Annular Double Bond.

The calculated contributions of the annular double bond of sterol A to the molecular rotation differences (M.R.D.) between saturated and unsaturated derivatives correspond to the values given by Barton for any nuclear double bond. The M.R.D. values calculated for sterol A are in the same direction and magnitude as those for the 8(14) position[15] (Table II).

Barton[15-19] showed that the molecular rotation differences between unsaturated sterols and their derivatives are characteristic of the position of the double bond in the molecule. The contribution of any double bond to the optical rotation of the steroid is independent of a second double bond if the double bonds are not conjugated and are

separated by at least three carbon atoms from other functional groups.

Thus if the contribution to the optical rotation of the sterol is known for the side chain double bond, it is possible to subtract this value from the molecular rotation, $[M]_D$, of the sterol and determine the contribution of an annular double bond in the molecule. Since the contribution of the second double bond is characteristic of its position, this method allows the position of the double bond to be determined.

The molecular rotations of a number of 3β-hydroxy-24β-ethyl-(5α)-cholesta-7,22-diene derivatives are listed below.

	$[M]_D$		
	alcohol	acetate	benzoate
parent 7,22-diene	-12	-23	+10
22;23 double bond contribution[16]	-61	-61	-61
Calculated $[M]_D$ for 7:8 unsaturated stenol	+47	+38	+71
Literature $[M]_D$[18]	+46	+36	+67

The M.R.D. (Δ values) may be calculated by subtracting the $[M]_D$ of the stenol* from the $[M]_D$ of the derivative:

$[M]_D$ derivative - $[M]_D$ stenol = M.R.D. (Δ value)

substituting the $[M]_D$ values for the above compounds

+38 (acetate) - 47 (stenol) = -9 (Δ_1)

+71 (benzoate) - 47 (stenol) = +24 (Δ_2)

*The molecular rotation of the stenol may be calculated by subtracting the contribution of the side chain double bond from the $[M]_D$ of the doubly unsaturated sterol.

TABLE II

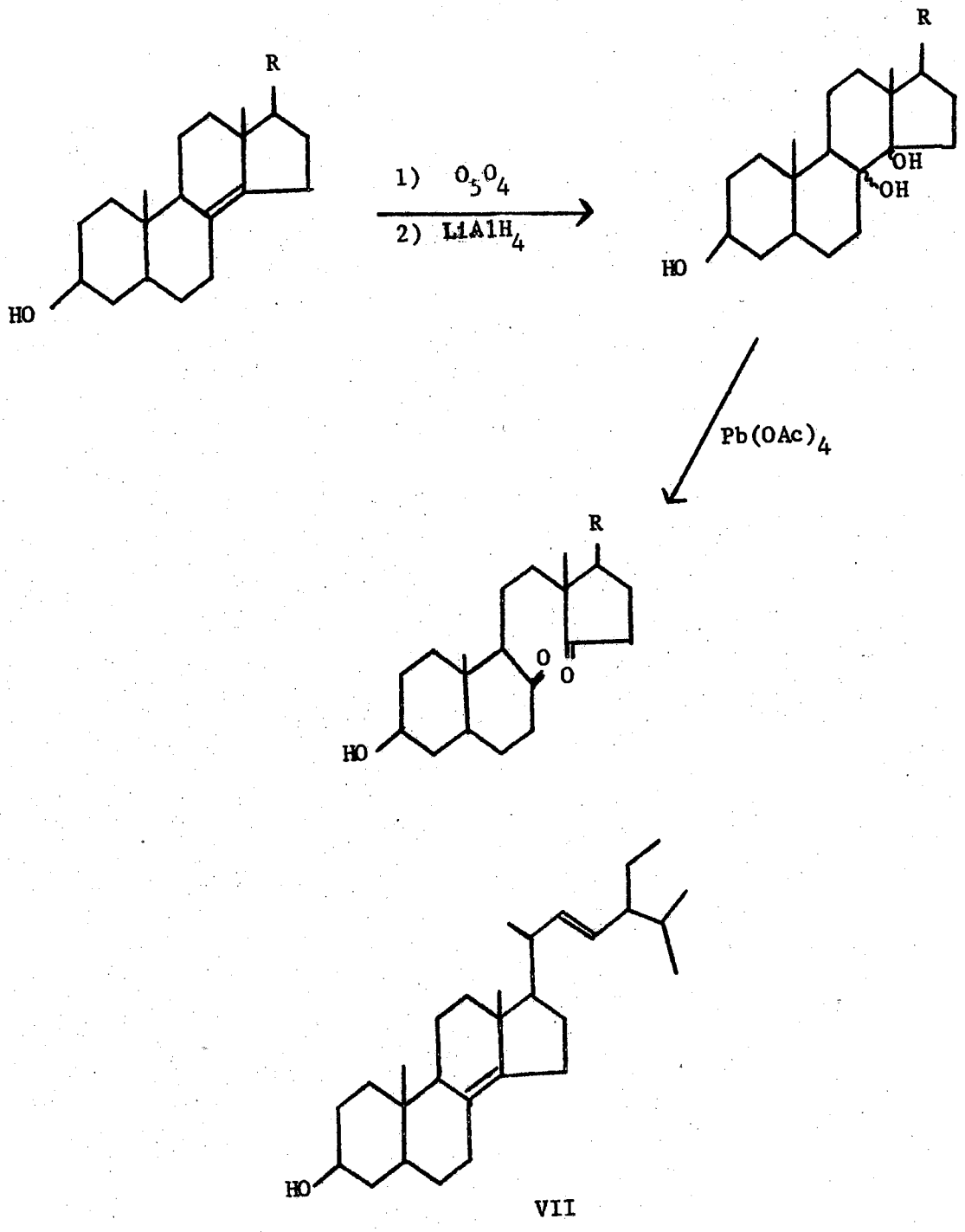
MOLECULAR ROTATION DIFFERENCES OF STENOL A

	$[M]_D$			
Substance	alcohol	acetate	benzoate	ketone
Sterol A	-37	-85	-104	+45
contribution of 22(23) double bond[16]	-61	-61	-61	-61
calculated value for stenol A	+24	-24	-43	+106

	M.R.D		
Substance	Δ_1 (acetate)	Δ_2 (benzoate)	Δ_3 (ketone)
Stenol A	-48	-67	+62
8(14)-stenols[15]	-40	-42	+100
8(9)-stenols[15]	+15	---	---
7-stenols[15]	-6	+30	+90

Since impurities can change optical rotation significantly, a chemical proof was needed. It was known from the N.M.R. spectrum of the sterol that the annular double bond must be tetra-substituted. To decide between the 8(14) and 8(9) positions, the osmium tetraoxide method developed by Castells[21] et al. was used according to the following scheme. (Fig. 1) The sterol was treated with osmium tetraoxide and the osmic ester reduced with lithium aluminum hydride. The glycol so obtained was oxidized to the diketone with lead tetraacetate.

The I.R. spectrum of the diketone is indicative of the position of the double bond in the sterol. Double bonds at the 8(9) position yield a ten-membered cyclic diketone, the I.R. spectrum of which shows only one carbonyl band; 8(14) double bonds yield a five-and a six-membered



VII

Figure 1. Degradation of Sterol A.

cyclic ketone, the I.R. spectrum of which contains two carbonyl bands.

The nuclear fragment from the ozonolysis of the sterol was used in this method to avoid interference from the side chain double bond. Ozonolysis of the sterol cleaves the side chain but leaves the nuclear double bond intact and destruction of the ozonide with zinc and water would not be expected to isomerize the remaining double bond. The carbonyl present in the nuclear ozonolysis fragment was reduced to the alcohol during the destruction of the osmic ester and offered no interference. The alternative method of hydrogenation of the side chain to avoid interference is not satisfactory since there is a possibility that the nuclear double bond might migrate even under "neutral" hydrogenation conditions. The ozonolysis fragment when subjected to the osmium tetraoxide process gave a doublet (1734 and 1725 $cm.^{-1}$) in the I.R. which proves the annular double bond in sterol A is at the 8(14) position. Thus sterol A is 3β-hydroxy-24β-ethyl-(5α) cholesta-8(14), 22-diene VII.

C. Experimental

Isolation of Sterol A.

Isolation of sterol A by partition chromatography on Florisil is described in chapter I.

Isolation of Sterol A via Girard's T-reagent. Physical Properties and Derivatives.

The non-ketonic fraction of "tremetol" (180 g., 65%) which had been obtained by Burke[12] from the treatment of "tremetol" with Girard's T-reagent was divided into four equal parts and chromatographed on

activity II alumina (30 ml. H_2O/kg., Merck acid-washed, 400 g. per run). The fractions were combined into four main divisions on the basis of solvent polarity.

TABLE III

ALUMINA CHROMATOGRAPHY OF NON-KETONIC FRACTION OF "TREMETOL"

Fraction	solvent	ml./run	combined wt.
1	ligroin	1000	66.5 g.
2	benzene	1000	29.8
3	chloroform	1000	43.9
4	methanol	1000	22.4
			162.6 g. (90% recovery)

Fraction 2 when rechromatographed on activity I alumina yielded several fractions of oil (11.5 g.:chloroform:benzene, 3:1) which when washed with methanol precipitated 6.1 g. of crude sterol A. Similar chromatography of fraction 3 yielded a dark oil (11 g.; chloroform) which when taken up in methanol and chilled overnight gave 3 g. of a saturated (no tetranitromethane test) hydroxy-ketone (m.p. 126-9°, crude) but no sterol A. Crude sterol A was isolated in 2.2% yield from "tremetol" by this method. Sterol A when crystallized from acetone-methanol, then acetone and dried (P_2O_5, under vacuum, 75°) overnight had m.p. 160-2°. Anal. Calcd. for $C_{29}H_{48}O \cdot CH_3OH$: C, 81.01; H, 11.79%. Found: C, 81.64; H, 11.51%. However crystallization from acetone-methanol or sublimation gave m.p. 152-4°, $[\alpha]_D$-9° (chloroform, c 0.0428). The acetate prepared by refluxing the sterol in acetic

anhydride for one hr. had m.p. 176.5-9°, $[\alpha]_D^{25}$-18.9° (chloroform, c, 0.0414). The benzoate prepared by refluxing the sterol with benzoyl chloride in pyridine for two hr. had m.p. 191-3° (ethyl acetate-methanol), $[\alpha]_D^{25}$-24.6° (chloroform, c, 0.00495).

Oppenauer Oxidation of Sterol A.

Crude sterol A (0.643 g.) was dissolved in dry toluene (150 ml.) containing freshly distilled cyclohexanone (15 ml.). To this solution was added a suspension of aluminum isopropoxide (1 g., Eastman practical) in toluene (100 ml.). The mixture was refluxed overnight, cooled and a concentrated solution of potassium sodium tartrate (15 ml.) added. The mixture was then steam distilled until no more organic material was present in the distillate. The residual suspension was filtered and the precipitate was taken up in chloroform. After drying over magnesium sulfate, the chloroform solution was evaporated and the solid residue crystallized from acetone-methanol to give 0.238 g. of crude ketone m.p. 80-100°. Several crystallizations from acetone-methanol followed by sublimation gave m.p. 174-6°, $[\alpha]_D$+11.1 (chloroform, c, 0.0556). Anal. Calcd. for $C_{29}H_{46}O$: C, 84.81; H, 11.29%. Found: C, 85.01; H, 10.68%.

Ozonolysis of the Side Chain.

All ozonolyses were carried out using a Welsbach T-23 ozonator. A typical example is given. A solution of sterol A (0.5 g.) in dry methylene chloride (100 ml.) was ozonized at -70° until the appearance of a blue color indicated the solution was saturated with ozone (about 3 hr.). The solution of the ozonide was allowed to warm to room temperature whereupon zinc and water were added. The mixture was stirred

overnight then steam distilled. The organic residue from the steam distillation was taken up in chloroform. Evaporation of the chloroform solution after drying over magnesium sulfate gave a yellow viscous oil which was later investigated (see below). The organic distillate from the steam distillation was dried over magnesium sulfate and the methylene chloride removed under reduced pressure to yield 0.070 g. of yellowish oil. Attempts to prepare 2,4-dinitrophenylhydrazone and semicarbazone derivatives of the oil were unsuccessful. However the amide could be obtained from the corresponding acid (see below).

Preparation of Amides from Steam-Volatile Ozonolysis Products.

The 2,3-dimethylbutyraldehyde (0.05 g.) obtained from ozonolysis of ergosterol was stirred with Tollens reagent (10 ml., 10%) for 4 hr. The ammoniacal solution was extracted with ether to remove any excess aldehyde, then acidified with dil. hydrochloric acid. The acidic solution was filtered and the filtrate extracted with ether. This ether extract after drying with magnesium sulfate was evaporated to yield 0.03 g. of the corresponding acid. The acid was treated with 1.5 times its volume of thionyl chloride for 0.5 hr. at 60°. The mixture was taken up in dry benzene (25 ml.) and chilled to -5°. Ammonia gas was bubbled through the solution for 15 min. After removing ammonium chloride by filtration, the benzene was evaporated at reduced pressure and the solid residue extracted with chloroform. Evaporation of the chloroform after filtering the suspended solids yielded 0.1 g. of white solid, m.p. 80-100°, after one crystallization from ligroin. Sublimation of the amide gave m.p. 124-5° (sealed capillary). Similar treatment of the volatile ozonolysis product

of sterol A gave m.p. 131° after sublimation.

Preparation of 2-ethyl-3-methylbutyramide.

Ethylisopropylmalonic acid, m.p. 130-5°, obtained from the saponification of diethyl isopropylethylmalonate (b.p. 60°/0.1 mm.) was heated slowly to 135°. After carbon dioxide evolution ceased, the acid was distilled at 190° under reduced pressure to yield 2-ethyl-3-methylbutyric acid. The amide prepared as above had m.p. 136° after sublimation.

Comparison of the Amides. Thin Layer Chromatography and Mixed Melting Points.

Thin layer chromatography of the 2-ethyl-3-methylbutyramide, sterol A-amide, and 2,3-dimethylbutyramide methylamides simultaneously on silica gel G (250 μ) using benzene: ether(9:1) and developing with iodine gave Rf values of 0.67, 0.66, and 0.57 respectively.

TABLE IV

MIXED MELTING POINTS

Mixed m.p.		Observed m.p.	Mixed m.p.	lit. m.p.
135-6°	2-ethyl-3-methylbutyramide	136°		136° [24]
	Sterol A amide	131°	126-7°	---
125-6°	2,3-dimethylbutyramide	124-5°		131° [25]

Degradation of Sterol A to Stigmastane.

Hydrogenation of Sterol A Acetate

Sterol A acetate (0.730 g.) in chloroform (75 ml.) was hydrogenated using a platinum oxide catalyst (0.125 g.) until hydrogen absorption ceased. Removal of the catalyst and solvent and chromatography of the residue on alumina gave 0.70 g. of a crystalline solid, m.p. 104-10°, $[\alpha]_D$ 12.5° (chloroform) which gave positive tetranitromethane and Tortelli-Jaffe tests, but whose infrared spectrum no longer possessed the C = C band at 970 $cm.^{-1}$ This dihydroacetate (0.374 g.) was dissolved in glacial acetic acid (40 ml.) containing perchloric acid (60%, 1 ml.) and hydrogenated over platinum oxide (0.260 g.) until hydrogen absorption ceased. After removal of the catalyst, the solution was poured into five times its volume of cold water and filtered. The precipitate was washed with water and dissolved in ethyl ether. After washing with sodium carbonate solution and water, and drying over magnesium sulfate, the solvent was evaporated to give 0.296 g. of the crude saturated sterol acetate (stanol acetate), which gave a negative tetranitromethane test. An additional 0.041 g. of crude stanol acetate was recovered from the aqueous acetic acid filtrate to give a total of 0.337 g. (90%). Saponification of the crude acetate (0.270 g.) in alcoholic potassium hydroxide yielded 0.266 g. of the saturated sterol (stanol A).

Oxidation of Stanol A.[26]

Crude stanol A (0.208 g.) was dissolved in glacial acetic acid (30 ml.) and chromic anhydride was added slowly to the solution. The mixture was stirred for 75 min. at room temperature, poured into

a large volume of cold water and filtered. The precipitate was washed with water and dissolved in ethyl ether. After washing with sodium carbonate solution and water, and drying over magnesium sulfate the solvent was removed by evaporation to yield the crude ketone. A small additional amount of ketone was recovered from the aqueous acetic acid filtrate. Total recovery of crude product was 0.19 g., 90%.

Reduction of Stanol A Ketone.

The crude ketone (0.150 g.) from the above oxidation was added to diethylene glycol (25 ml) containing potassium hydroxide (1.5 g.) and 95% hydrazine hydrate (1.5 ml.). The solution was refluxed for 7 hr., additional diethylene glycol (1.5 ml.) and hydrazine hydrate (0.5 ml.) added and the solution refluxed for 4 hr. The alkaline solution was poured into cold water and the aqueous suspension extracted with ethyl ether. The ether solution after washing with water and drying over magnesium sulfate was evaporated under reduced pressure to give a blackish oil which upon alumina chromatography yielded 0.70 g. of a hydrocarbon, m.p. 85° (methanol). Comparison of the hydrocarbon with cholestane, ergostane, and stigmastane, prepared similarly from the parent sterols, by vapor phase chromatography showed it to be identical to stigmastane.

Vapor Phase Chromatography of the Steroid Hydrocarbons.

The experiments were carried out with an Aerograph A-500 gas chromatograph using nitrogen as the carrier gas, a hydrogen flame ionization cell detector, an Aerograph model 500 electrometer and a

Leeds Northrup Speedomax H strip chart recorder. Data for the two columns used are given in Table V. The columns will be referred to by the numbers given in the table.

TABLE V

GAS CHROMATOGRAPHY OF STEROID HYDROCARBONS

Column
I. 5% SE-30 on acid-washed Chromosorb W, ¼" x 5', stainless steel.
II. 2% QF-1 on Chromosorb W (HMDS), 80-100, ¼" x 6', aluminum.

Combined injections of any pair of the known hydrocarbons, cholestane, ergostane, and stigmastane, on both columns demonstrated that any pair could be resolved. The unknown hydrocarbon was identified by injecting a combined sample of it and each of the knowns and simply observing whether there were one or two peaks on the chromatogram.

Compound	Column I (57 ml./min.) RT (277°)	Column II (40 ml./min.) RT (200°)	RT (216°)
Cholestane			4 min.
ergostane	4 min.	5 min.	
stigmastane	5	6.5	
hydrocarbon A	5	6.5	5.5

Determination of the Position of the Annular Double Bond in Sterol A.[20]

The nonvolatile fragment of the sterol obtained from ozonolysis was treated with diazomethane in ether. The methyl ester (0.138 g.) was dissolved in pyridine (4 ml.) containing osmium tetraoxide (0.25 g.) and the mixture refluxed for 1 hr. The solvent was removed at 100° under reduced pressure and the residue taken up in dry tetrahydrofuran

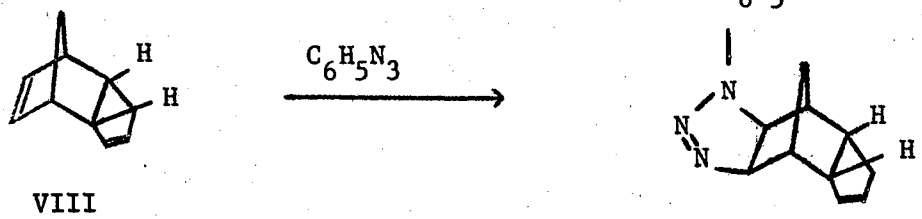
(10 ml.). Lithium aluminum hydride (0.5 g.) was added to the mixture and the suspension refluxed for 0.5 hr. Excess lithium aluminum hydride was destroyed with ethyl acetate and water and the solvent evaporated at 100° under reduced pressure. The residue was extracted with ethyl ether and the ether solution dried over magnesium sulfate. Evaporation of the ether gave an oil, the infrared spectrum of which showed only a few per cent carbonyl absorption. The oil was dissolved in glacial acetic acid (5 ml.) containing some t-butyl alcohol (0.5 ml.) to prevent acetate formation. Lead tetraacetate (0.250 g.) was added and the solution stirred at room temperature for 10 hr. After evaporation of acetic acid under reduced pressure the residue was partitioned between water and ethyl ether. The ether solution was washed with water, sodium bicarbonate solution, and water. After drying over magnesium sulfate the ether solution was filtered through activity III alumina and solvent evaporated to give 0.08 g. of colorless oil the infrared spectrum of which possessed a carbonyl doublet with maxima at 1734 and 1725 $cm.^{-1}$. Further treatment of the oil with lead tetraacetate for 12 hr. at room temperature gave an oil in which the same doublet was more clearly defined.

CHAPTER III

THE REACTION OF VARIOUS AZIDES WITH UNSATURATED COMPOUNDS

A. Historical and Introduction

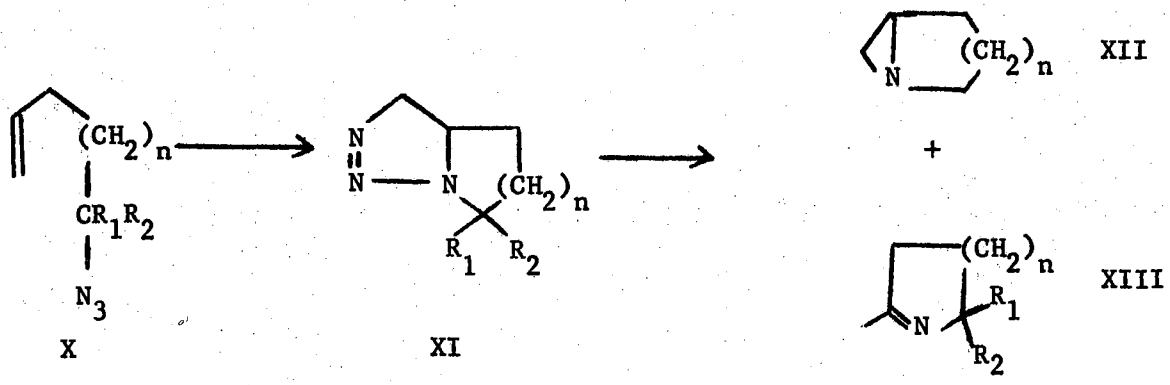
It has been known for over fifty years that phenyl azide and acetone react when heated in a sealed tube to give 1-phenyltriazole.[26] Later it was found that phenyl azide reacted with many bicyclo(2.2.1)-heptene derivatives to form the corresponding 1:1 dihydrotriazole adducts.[27] Since only strained alkenes were found to react readily with phenyl azide, the reaction soon became a diagnostic test for strained double bonds. In the reaction of dicyclopentadiene (VIII) with phenyl azide,[27] the product is the mono-adduct IX in which only the strained double bond has reacted.



VIII IX

Although organic azides usually react with strained alkenes or double bonds activated by functional groups, Logothetis[28] has recently demonstrated that it is possible to design unsaturated organic azides (X)

which will undergo an intramolecular addition to form dihydrotriazoles (XI) from a non-strained double bond.



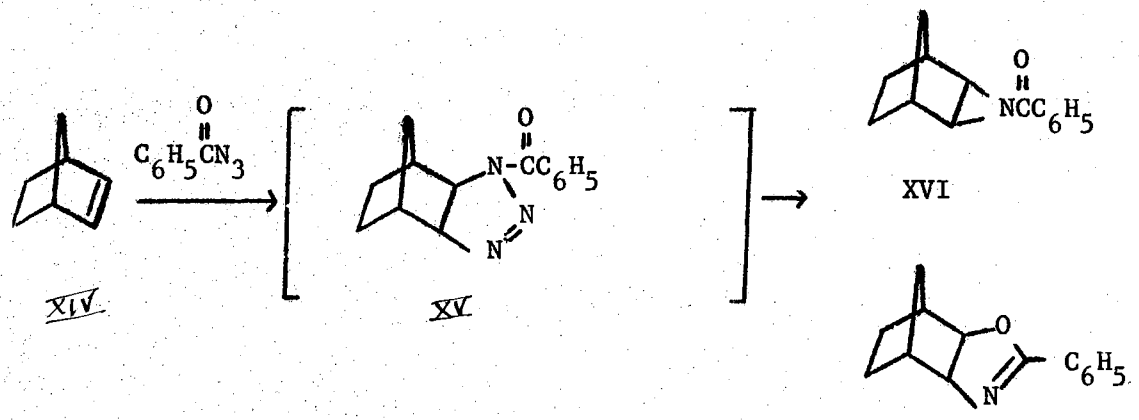
Recently, Huisgen and his associates[29] have illustrated that this reaction is only one member of a large group of reactions which they refer to as 1,3-dipolar cyclo additions. Evidence has been presented that these 1,3-dipolar additions proceed through a polycentered concerted mechanism rather than a stepwise ionic addition.[30,31]

The phenyl dihydrotriazole adducts in the bicycloheptene series are relatively stable; only when heated to about 150° C do they lose nitrogen to give mixtures of aziridines and imines.[27] However when azides containing adjacent electron-withdrawing groups such as picryl,[32] benzoyl,[33] p-toluenesulfonyl,[34] and benzenesulfonyl[35,36,37,38,39] react with bicyclic olefins, the dihydrotriazole is usually difficult or impossible to isolate; instead the products correspond to 1:1 adducts minus molecular nitrogen.

Brunner[34] treated p-toluenesulfonyl azide with strained alkenes

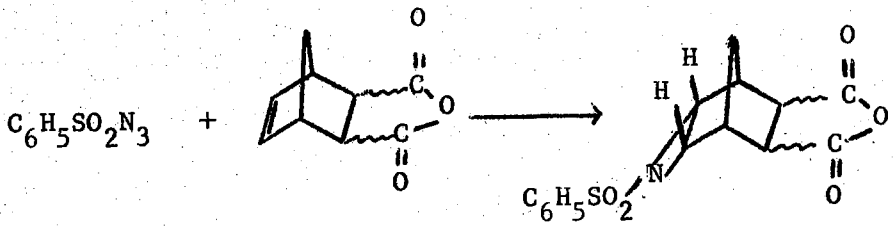
such as dicyclopentadiene and norbornene and found the products had analyses corresponding to the 1:1 addition products minus molecular nitrogen. Although he offered no chemical evidence for the structures of these compounds, he suggested the dicyclopentadiene derivative possessed the sulfonimide structure.

Huisgen[33] reported that the addition of benzyl azide to norbornene (XIV) is followed even at 40° C by elimination of nitrogen from the dihydrotriazole XV, which apparently was not isolable, to give the N-benzylaziridine XVI and the oxazoline XVII, but he provided no supporting evidence for the intermediacy of XV.

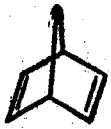


XIV XV XVI XVII

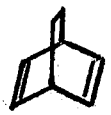
Other work in this laboratory has shown that benzenesulfonyl azide reacts with bicyclo(2.2.1)-5-heptene-endo-cis-2,3-dicarboxylic anhydride (XVIII) and its exo-analogue (XXIX) in refluxing carbon tetrachloride to give, surprisingly, the endo-aziridines (XX or XXI).[35,37]



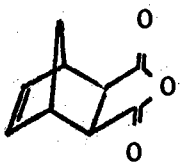
XVIII endo
XXIX exo
XX exo
XXI endo



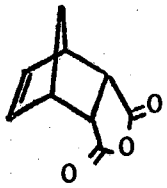
XXII



XXIII

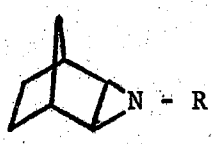


XXVIII

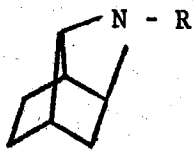


XXIX

Concurrent with the present investigation, other work in this laboratory has shown the course of the reaction of benzenesulfonyl azide with dicyclopentadiene (VIII)[36,38], bicyclo(2.2.1)-2,5-heptadiene (XXII)[36,38], bicyclo(2.2.2)-2-octene (XXIII)[36], and bicyclo(2.2.1)-2-heptene (XIV)[38,39] to give predominantly aziridine products.



XXIV



XXV

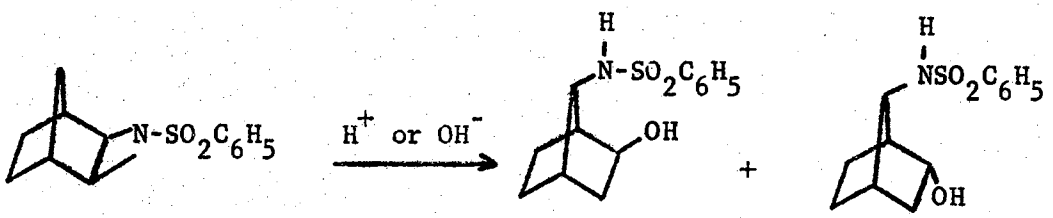
The present investigation was undertaken to 1) study the course of the reaction of bicyclo(2.2.1)-2-heptene with various organic azides; 2) establish the structure of the addition products; 3) study the reactions of the addition products.

B. Results and Discussion

Structures of the 1:1 Adducts of Bicyclo(2.2.1)-2-heptene and Benzenesulfonyl Azide, its p-Methoxy, p-Bromo, and p-Nitro Derivatives and Benzoyl Azide.

The p-methoxy, p-bromo, and p-nitro derivatives of benzenesulfonyl azide were prepared and reacted with norbornene (XIV). All three reactions were exothermic and nitrogen was eliminated spontaneously. The observation was made that the evolution of nitrogen was surface-dependent since rapid stirring resulted in a rapid evolution of nitrogen. The solid products usually crystallized out overnight.

Previous workers in this laboratory had established that the parent benzenesulfonyl azide adduct possessed structure XXIVc or XXVc[39].



XXIVc XXX XXXI

The n.m.r. spectra of the p-substituted adducts under study (see table VI) were strikingly similar to that of the parent, but the singlet assigned to the protons under nitrogen was shifted and showed no signs of splitting. This suggested that the adducts were in fact of the 2,3-aziridine type, since it would be unreasonable to expect the protons at the 2- and 7-positions to be identical in widely differing environments. For a further comparison, the preparation of the benzoyl

TABLE VI

CHEMICAL SHIFTS (p.p.m.) OF AZIDE-NORBORNENE ADDUCTS

Substituent	H-2, H-3	H-1, H-4	Solvent
(XXIVa) p-$O_2NC_6H_4SO_2$-	3.12	2.50	$CDCl_3$
(XXIVb) p-$BrC_6H_4SO_2$-	3.00	2.49	$CDCl_3$
(XXIVc) $C_6H_5SO_2$-	2.84	2.42	CCl_4
XXIVd p-$CH_3OC_6H_4SO_2$-	2.73	2.35	CCl_4
(XXIVe) C_6H_5CO-	2.61	2.48	CCl_4
(XXIVd) $C_6H_5CH_2$-	1.50	2.32	CCl_4

adduct reported by Huisgen[33] was undertaken. In our hands no dihydrotriazole intermediate could be isolated; the aziridine XXIVe and the oxazoline XVII were isolated directly. The n.m.r. spectrum of XXIVe was found to be remarkably similar to the spectra of the other azide adducts. A direct correlation of XXIVe with XXIVc was made in the following manner.

Removal of the benzoyl group from XXIVe to give the amine XXVI was accomplished by inverse addition of lithium aluminum hydride by a known procedure.[40] Treatment of XXVI with benzoyl chloride gave back XXIVe, identified by i.r. and n.m.r., and treatment of XXVI with benzenesulfonyl chloride gave XXIVc, identified by mixed m.p. with authentic sample.

Even though the correlation of the adducts was established, the possibility that all were of the azetidine type XXV still existed since Huisgen had not offered any evidence in support of the aziridine structure for XXIVe. Accordingly the previously unreported benzyl dihydrotriazole was prepared and its structure conclusively established in the

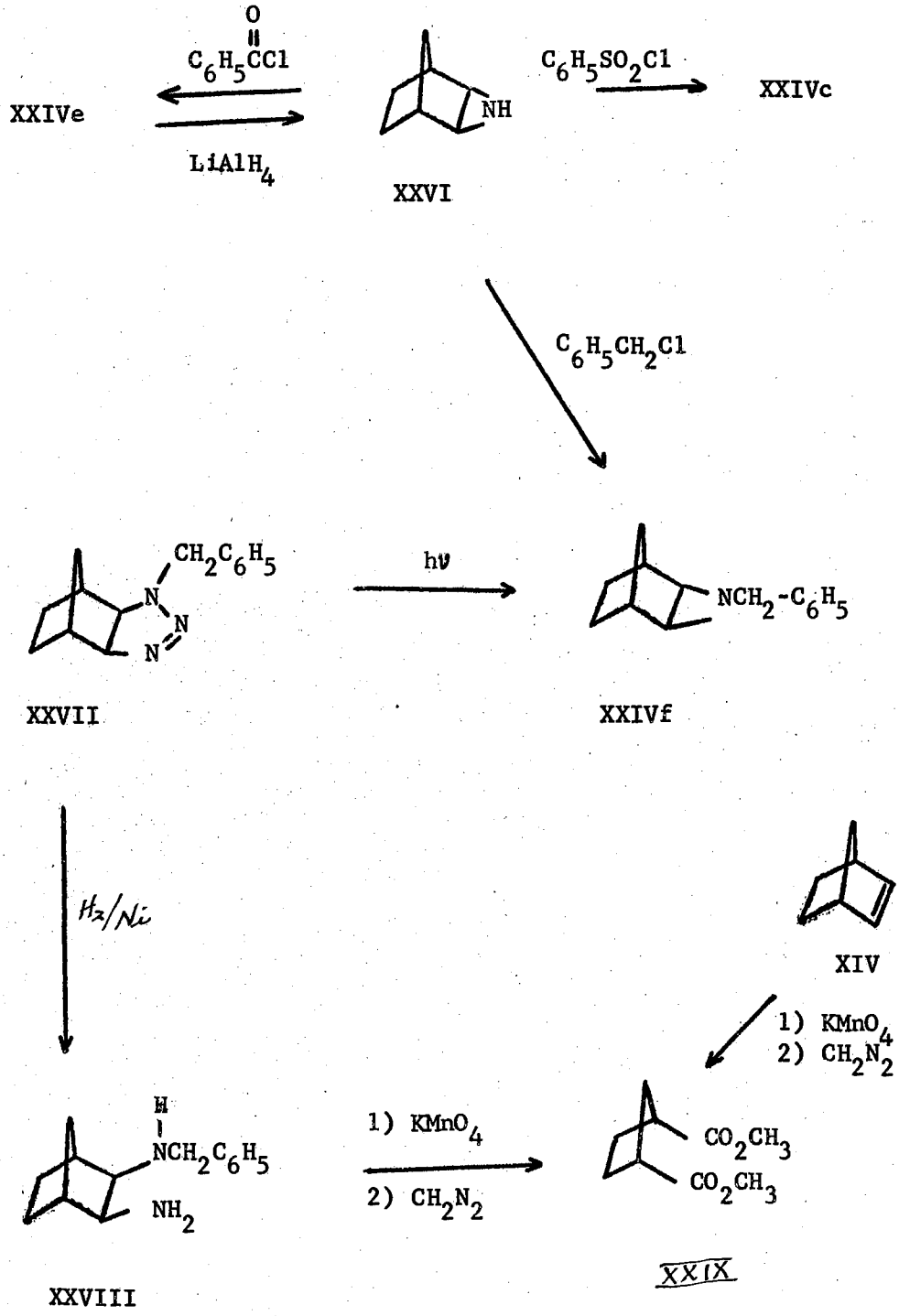


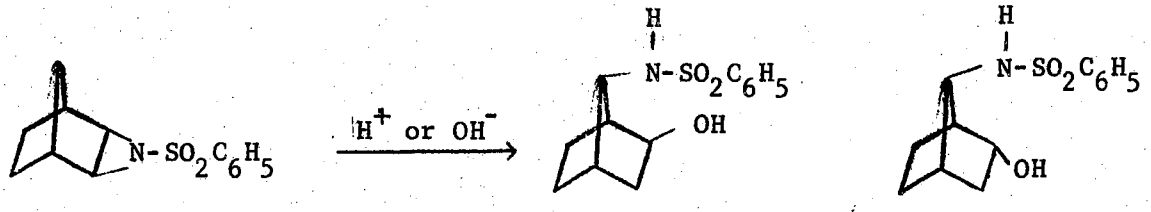
Figure 2. Structure Proof of Azide-Norbornene Adducts.

following manner.[38]

Hydrogenation of XXVII gave the diamine XXVIII which was oxodized with permanganate to the diacid. The diacid was identified as the dimethyl ester XXIX by v.p.c. comparison of an authentic sample prepared from oxidation of XIV. A 2,7-adduct could not have given this acid. Photolysis of XXVII gave the aziridine XXIVf which was correlated with the other adducts by treatment of XXVI with benzyl chloride to give XXIVf. Rearrangement during photolysis is unlikely since the norbornyl radical is known to be resistant to rearrangement.[41,42,43] Recently, Franz and Osuch[44] have also reported chemical evidence in support of structure XXIVc.

The Acid-Catalyzed Hydrolysis of XXVII.

The acid-catalyzed hydrolysis of a number of bicyclic dihydrotriazoles had been reported by Alder and co-workers[45,46] to give only the corresponding 2,3-amino-alcohols. Triazolines of norbornene itself were not included in this work. In light of the fact that hydrolysis of XXIVc gives rise to a mixture of the 2,7-rearrangement products[39], it was decided to study the acid-catalyzed hydrolysis of XXVII to determine if any aziridine was formed and to determine the ratio of products.



XXIVc XXX XXXI

The following scheme (Figure 3) represents the possible reaction paths.

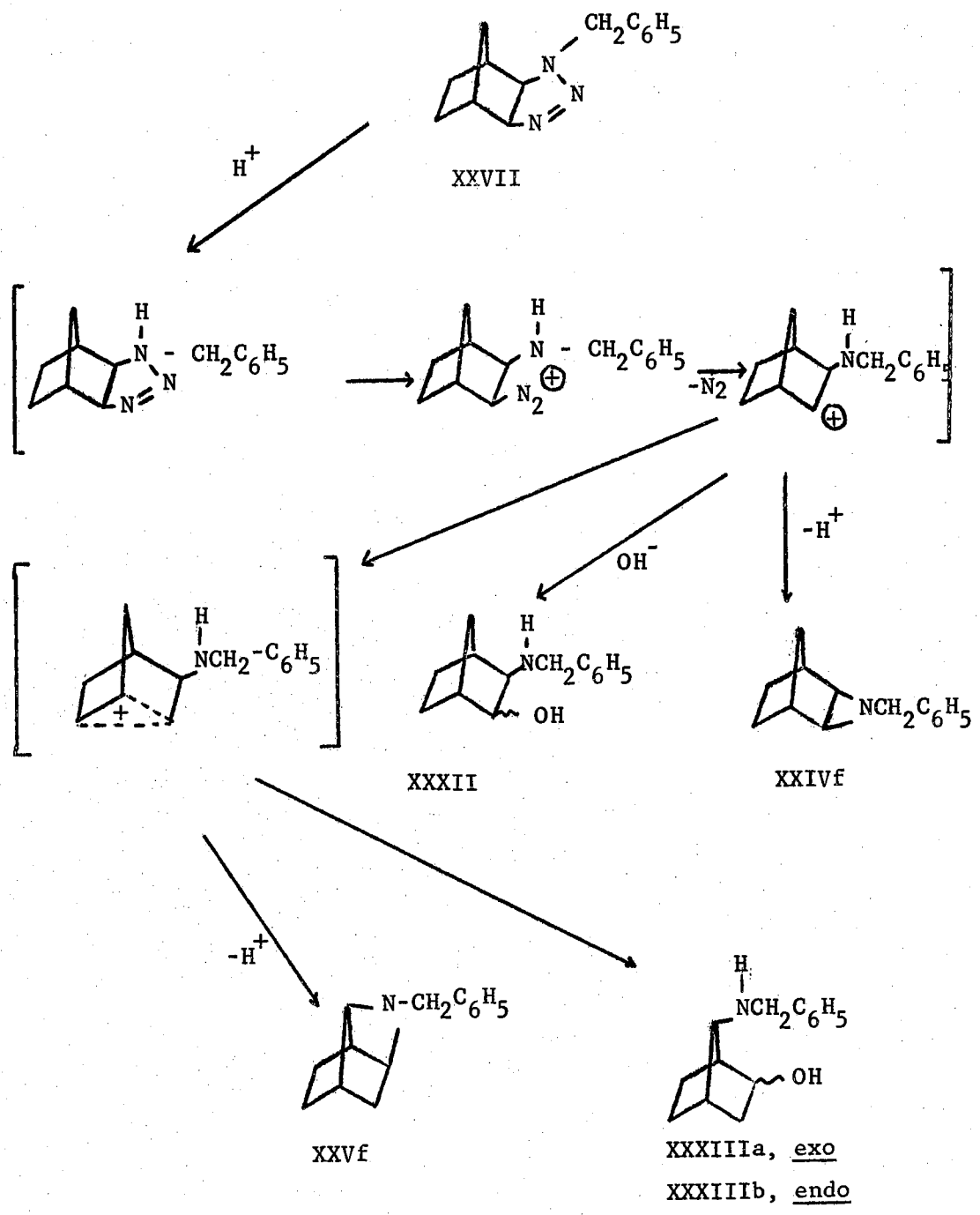


Figure 3. Possible Reaction Paths of Acid-Catalyzed Hydrolysis of XXVII.

Recently Huisgen[47] has reported on the decomposition of the triazoline, XXXIV, the adduct of phenyl azide and methyl propenate. Upon heating at 85°, XXXIV yields the aziridine XXV. However when XXXIV is hydrolyzed with triethylamine, it yields methyl 3-anilino-2-diazopropanote, XXXVI. When XXXVI is heated to 100° it forms only the eneamine XXXVII. Huisgen points out that thermolysis of XXXIV cannot go through the same transition state as that of XXXVI since the products and half-lives of the two thermolysis are different. In addition, Huisgen studied the decomposition in solvents of varying polarity and found little change in rate with change of solvent. He feels that the evidence casts doubt on the dipolar nature of the triazoline decomposition intermediates as proposed by him[33], (XXXVIII and XXXIX), and Logothetis[28] earlier. However he emphasizes that these conclusions may only be valid for this series. Obviously the acid hydrolysis of triazolines is an extremely different case. It is hard to conceive of a proton adding to any but the benzyl nitrogen to give the intermediates we propose.

The acid catalyzed hydrolysis of XXVII with HCl in the manner of Alder and co-workers[45,46] gave at least six products as shown by thin layer chromatography (TLC). Three major products of the hydrolysis have been isolated in pure form by column chromatography; a fourth relatively major component has not been isolated in pure form. The exact relative percentages of the three major components were difficult to estimate since some fractions from the chromatographed were contaminated.

The three major components are the aziridine XXIV (25%), identified by n.m.r. and i.r. comparison with an authentic sample, and two amino alcohols B and C (10%). Preliminary evidence indicates that the two

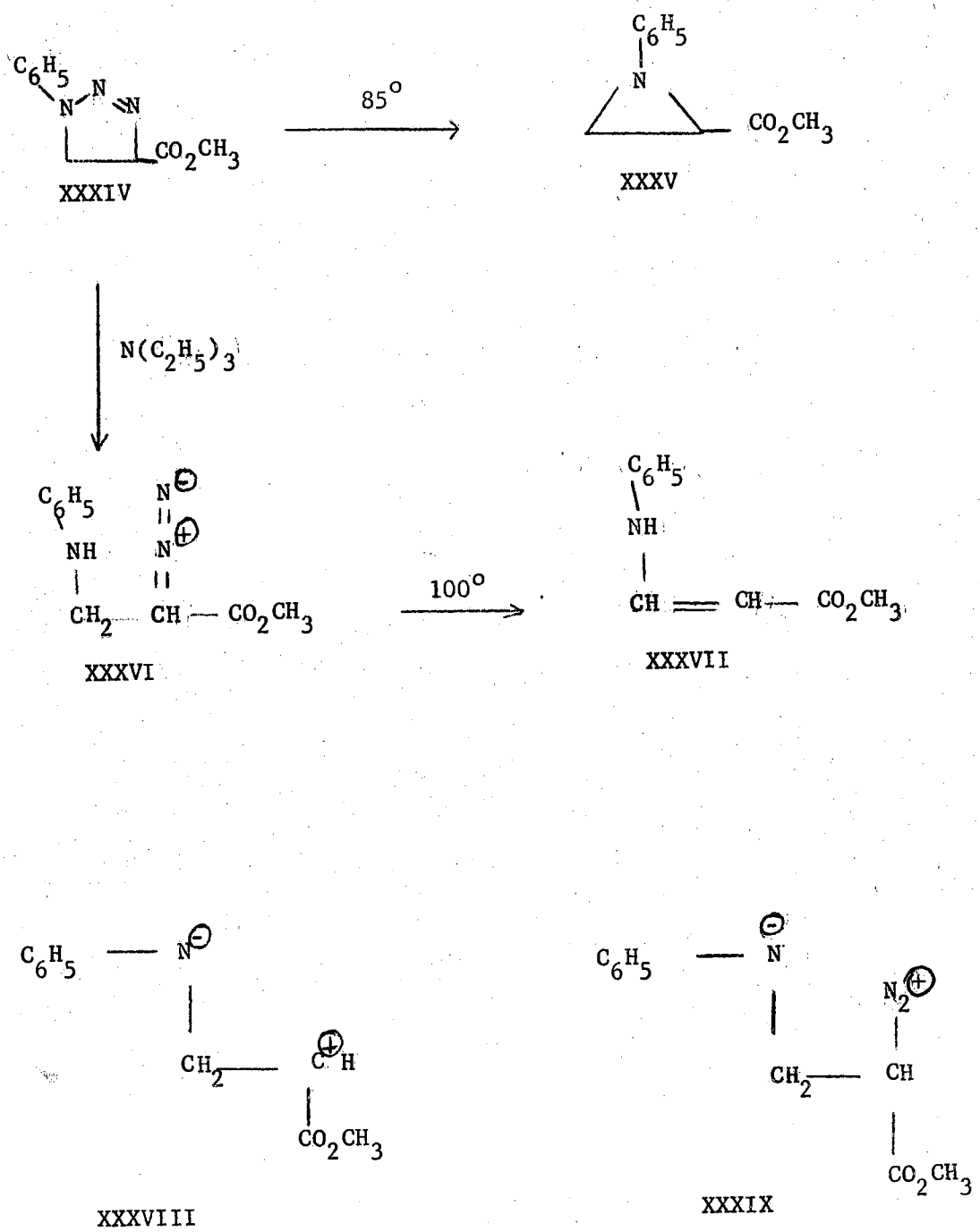


Figure 4. Decomposition of Triazoline XXXIV.

amino alcohols may be the 2,7-rearrangement products, XXXIIIa and XXXIIIb (see below). No product corresponding to the azetidine XXIVf was isolated.

It is suggested that the aziridine and the amino alcohols are formed from a common carbonium ion intermediate. The possibility that the amino alcohols arise from further hydrolysis of a first-formed aziridine was ruled out, since the treatment of the aziridine under conditions similar to that of hydrolysis of the triazoline results in only a slight hydrolysis of the aziridine.

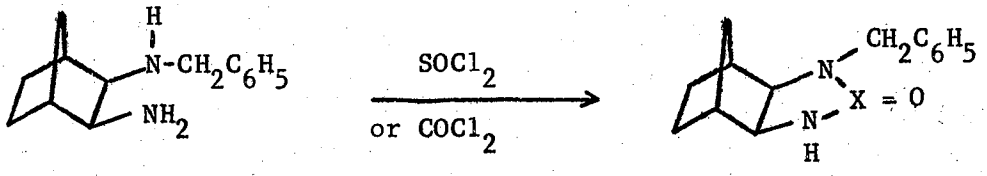
The n.m.r. spectrum of the amino alcohols and their corresponding diacetates showed the following signals in chloroform containing D_2O which were tentatively assigned on the basis of evidence presented below: Amino alcohol B: δ 3.78 (H-2 and two benzylic protons), 2.95 (H-7), the signal at δ 3.78 is resolved in benzene to give a broad multiplet δ 3.61-3.90 (H-2) and a singlet at δ 3.5 (two benzylic protons); the signal for H-7 occurs at δ 2.61. Amino alcohol B diacetate: δ 4.70 (H-2, triplet, J=5 c.p.s.), 4.40 (two benzylic protons, doublet, J=9 c.p.s.), 3.45 (H-7), 3.18 (one tertiary proton), 2.5 (one tertiary proton). Amino alcohol C: δ 3.70 (H-2 and two benzylic protons), 3.20 (H-7). The signal at δ 3.70 could not be resolved in benzene, nitrobenzene, or carbon disulfide; only partial resolution could be obtained with thiophene. Amino alcohol C diacetate: δ 4.32-4.63 (H-2, multiplet), 4.21 (two benzylic protons), 3.89 (H-7), 2.55 (two tertiary protons, multiplet).

Tentative assignments of structure for B (XXXIIIa) and C (XXXIIIb) have been made on the following basis. In both cases the peak for the proton under nitrogen (H-7) is rather sharp while the peak for the

proton under oxygen (H-2) is a broad multiplet. If either B or C were a 2,3-amino alcohol, the proton under nitrogen would be expected to be a broad multiplet also.[19] Therefore, both compounds should be of the 2,7-type. It was thought that by making the diacetate, the steric repulsion between the benzyl group and the 3-exo-oxygen function should be increased and that the dihedral angle between the 2,3-protons should be decreased, thereby sharpening the multiplet due to the proton under oxygen. This occurred in the case of B where the multiplet sharpened to a triplet. In addition, the benzyl protons are split to a doublet. The tertiary protons are also widely separated, indicating that one proton is in a quite different environment from the other.

In the case of the diacetate of C, the multiplet due to the proton under oxygen (H-2), was better resolved from the benzyl protons (quartet) than in the parent; however, no splitting of the benzyl protons was observed and both tertiary protons appeared as a broad singlet.

Attempts to make a cyclic sulfite or cyclic urea of the model compound XXVIII failed.



XXVIII XXXIV

X = S or C

Jones oxidation products of B and C show identical benzyl protons and identical H-7 protons in their n.m.r. spectra, but the methylene regions are not superimposable. TLC of the products shows they are quite impure. A possible argument in favor of the fact that the two ketones are identical would be that one would expect the proton under

nitrogen for the 2,3 case to be in a quite different position from the 2,7 case.

C. Experimental

Melting points were taken on a Fisher-Johns apparatus and are uncorrected. Infrared spectra were recorded with a Beckman IR-5 spectrophotometer; n.m.r. spectra were obtained with the Varian A-60 n.m.r. spectrometer, using tetramethylsilane (TMS) as an internal standard (δ=0). Gas chromatographic comparisons were made with an Aerograph A-700 gas chromatograph using a thermal detector. Carbon and hydrogen analyses were performed by Midwest Microlabs, Inc., Indianapolis, Indiana. All thin layer chromatograms were developed in iodine vapor. Unless otherwise noted, the alumina used in column chromatography was acid-washed (Merck).

Preparation of Sulfonyl Azides.

The preparation of the azides used was a modification of the procedure worked out by C. D. Kennedy.[48] A typical example is given.

p-Bromobenzenesulfonyl chloride (50 g., 0.2M) in 70 cc. of dioxane was added to 13.5 g. of sodium azide in 38 ml. of water and 10 cc of dioxane over 15 minutes at 15-20° C. The mixture was stirred overnight; water was added and the fractions separated. The aqueous layer was extracted three times with ether (300 ml.) and the ether extract combined with the organic layer. The ether solution was washed three times with 10% sodium hydroxide (the last time for 1 hour), twice with water and dried over magnesium sulfate. Evaporation of the solvent at about 80° C under reduced pressure gave 42.9 g. (84%) of p-bromobenzenesulfonyl

azide, m.p. 52-3°.

p-Methoxylbenzenesulfonyl azide prepared as above had m.p. 48-51° (one crystallization from ether-ligroin).

p-Nitrobenzenesulfonyl azide prepared as above except that the sodium hydroxide wash was omitted gave pale yellow needles m.p. 80-90° in 77% yield. Recrystallization from dioxane-petroleum ether (b.p. 60-70°) gave yellow needles m.p. 98-100°.

Preparation of N-(p-methoxybenzenesulfonyl)-3-azatricyclo-(3.2.1.$0^{2,4}$-exo)-octane (XXIVd).

The method follows the procedure of Zalkow and Oehlschlager.[39] Norbornylene (14 g., Roberts Chemicals, b.p. 93°) in 2 cc. of benzene was added to a solution of p-methoxybenzenesulfonyl azide (13.69 g., m.p. 48-51°) in 20 ml. of benzene at 7° C with stirring. The solution was allowed to come to room temperature and stirred overnight. Evaporation of solvent and excess norbornylene at reduced pressure gave an oil which when washed twice with petroleum ether (b.p. 60-70°) gave a 16.61 g. (79%) of material m.p. 80-90°. The solid was crystallized 8 times from benzene-petroleum ether (60-70°) to give m.p. 105-8°; ν_{max}^{KBr} 1610, 1500, 1439, 1325, 1266, 1163, 848, 770, and 709 $cm.^{-1}$. N.m.r. (in CCl_4) δ 0.5-1.5 (six protons), 2.35 (two protons), 2.73 (two protons), 3.88 (three protons), 6.8-7.9 (four protons). Anal. Calcd. for $C_{14}H_{17}NO_3S$: C, 60.19; H, 6.13%. Found: C, 60.64; H, 6.29%.

Preparation of N-(p-Bromobenzenesulfonyl)-3-azatricyclo(3.2.1.$0^{2,4}$-exo)-octane (XXIVb).

Compound XXIVb prepared as described above was obtained as a solid m.p. 100-10°, in 83% yield. Six crystallizations from benzene-petroleum

ether (60-70°) gave m.p. 121-2°; ν_{max}^{KBr} 1626, 1562, 1313, 1160, 1084, 969, 909, 826, 775, and 763 cm.$^{-1}$, n.m.r. (in $CDCl_3$) δ 0.75 (one proton, doublet, J=10), 1.25-1.75 (five protons), 2.45 (two protons), 2.99 (two protons), 7.60-8.05 (four aromatic protons). Anal. Calcd. for $C_{13}H_{14}BrNO_2S$: C, 47.57; H, 4.30. Found: C, 47.77; H, 4.47.

Preparation of N-(p-Nitrobenzenesulfonyl)-3-azatricyclo(3.2.1.0$^{2,4\text{-exo}}$)-octane (XXIVa).

This aziridine was prepared as above except that dioxane was used as a solvent; XXIVa was obtained as a pale yellow solid, m.p. 135-45°, in approximately 75% yield. Six crystallizations from benzene-petroleum ether (60-70°) gave m.p. 147-8°; ν_{max}^{KBr} 1318, 1167, 980, 902, and 637 cm.$^{-1}$ N.m.r. δ 0.80, (one proton; doublet, J=10), 1.02-1.70 (five protons), 2.50 (two protons), 3.10 (two protons), 8.35-8.85 (four aromatic protons). Anal. Calcd. for $C_{13}H_{14}N_2O_4S$: C, 53.05; H, 4.79. Found: C, 53.50; H, 5.12.

Preparation of N-Benzoyl-3-azatricyclo(3.2.1.0$^{2,4\text{-exo}}$)octane (XXIVe).

Benzoyl azide was prepared as described below and used without further purification by distillation. Benzoyl azide (24.2 g.) was added to 22.7 g. of XIV in 10 ml. of benzene and the solution stirred at room temperature for 48 hrs. The solvent and excess XIV were removed under vacuum at room temperature. The n.m.r. spectrum of the crude oil was essentially the same as that for the purified compound and showed no evidence of the expected triazole XV. Distillation of the oil gave 22.0 g. (63%) of XXIVe, b.p. 112-118°/0.18 mm., ν max 1653, 1370, 1316, 1276, 704, and 654 cm.$^{-1}$; n.m.r. δ 0.80 (one proton, doublet, J = 10), 0.97-1.80 (five protons), 2.48 (two protons), 2.60 (two protons),

7.1-8.1 (five aromatic protons). Analytical sample of XXIVe had b.p. $106^{\circ}/0.003$ mm., n_D^{28} 1.5710. Anal. Calcd. for $C_{14}H_{16}NO$: C, 78.84; H, 7.09. Found: C, 78.93; H, 7.42. A solid residue (4.26 g., m.p. 110-14°) was also obtained. ν_{max}^{KBr} 1653, 1613, 1447, 1391, 827, 769, 704, and 685 $cm.^{-1}$ No solvent to permit n.m.r. study was found.

Preparation of 3-Azatricyclo(3.2.1.$0^{2,4\text{-exo}}$)octane (XXVI).

The procedure follows that of Micovic and Mihailovic.[40] A slurry of lithium aluminum hydride (0.300 g., 0.007 m.) in 60 ml. of dry ether was added to a stirred solution of crude XXIVe (5.560 g., 0.026 mole) in 80 cc. of dry ether over 0.5 hr. at 0° C. The mixture was stirred for an additional 1.5 hr. at 0° C. Water (0.5 cc.) was added to the cold mixture followed by 0.5 cc. of 10% sodium hydroxide and 0.8 ml. of water. The mixture was stirred for a few minutes at room temperature and the precipitate removed by filtration. The ether solution was chilled and extracted five times with cold 10% hydrochloric acid (100 ml.). The cold aqueous acid extract was made basic with cold concentrated sodium hydroxide solution and extracted four times with ether (200 ml.). The ether extract was washed twice with water and dried over magnesium sulfate. Evaporation of the solvent gave 2.5 g. (87%) of crude XXVI. ν max 3333, 1639, 1092, 1047, 848, 819, 758, and 694 $cm.^{-1}$ N.m.r. δ 0.58 (one proton, doublet, J=11), 1-1.67 (6.4 protons), 1.82 (1.7 protons), 2.24 (two protons). The crude material was used for further reactions without purification.

Preparation of 1-Benzyl-3a,4,5,6,7,7a-hexahydro-4,7-methano-IH-benzotriazole (XXVII).

Crude benzyl azide (59 g.) and 70 g. of norbornene in 10 ml. of

benzene were stirred overnight. Evaporation of the solvent and excess norbornene under vacuum gave 103 g. crude XXVII (99%). The analytical sample prepared by recrystallization from petroleum ether, had m.p. 72-73°; ν_{max}^{KBr} 1455, 1352, 1091, 948, 742, and 700 cm.$^{-1}$; n.m.r. (in CCl_4) δ 0.9-1.5 (six protons), 2.08 (H-4), 2.57 (H-1), 2.98 (H-2, doublet, $J^{2,3}$=9.8 c.p.s.), 4.25 (H-3, doublet, $J^{2,3}$=9.8 c.p.s.), 4.72 (two benzylic protons, doublet, J=4 c.p.s.), and 7.30 (five aromatic protons).

Anal. Calcd. for $C_{14}H_{17}N_3$: C, 73.97; H, 7.54. Found: C, 74.29; H, 7.70.

Preparation of N-Benzyl-2,3-norbornanediamine (XXVIII).

The benzotriazole XXVII (9.2 g.) in 95% ethanol was hydrogenated in the presence of 2 g. of Raney nickel under 40 p.s.i. on a Parr apparatus for two days. Removal of the catalyst by filtration and evaporation of the solvent under reduced pressure at about 80° gave an oil which was taken up in ether, washed with water and dried over magnesium sulfate. Evaporation of the ether gave 8.4 g. (96%) of XXVIII which was not purified further. ν_{max}^{KBr} 3279, 1605, 1439, 1342, 1152, 1117, 1089, 943, 885, 847, 741, and 699 cm.$^{-1}$; n.m.r. δ 0.61-2.18 (ten protons), 5.65 (two protons, AB quartet, $J^{2,3}$=10 c.p.s.), 3.76 (two benzylic protons) and 7.25 (five aromatic protons).

Preparation of cis-1,3-Cyclopentanedicarboxylic Acid Dimethyl Ester (XXIX).

From Norbornene.

The procedure used is a modification of that described by Birch[49]. Norbornylene (12 g.) in 100 ml. of ligroin was added to 200 ml. of water

in a three-necked flask. Dry ice was added at intervals while a 5% potassium permanganate solution was added dropwise with rapid stirring. Addition of the permanganate was discontinued after the mixture acquired a permanent purple color (after about 3 hours). The mixture was allowed to stir at 25° for an additional 3 hours; sulfur dioxide was passed into the mixture to remove the manganese dioxide and the mixture made strongly acidic with hydrochloric acid. The acidic mixture was extracted four times with ether (600 cc.), and the ether extract was washed twice with water, and dried over magnesium sulfate. Evaporation of the ether gave 4.5 g. of crude solid acid. The crude solid was taken up in 20% sodium carbonate solution and the solution extracted with ether. The aqueous solution was acidified with hydrochloric acid and extracted with ether. The ether extract was washed once with water and dried over magnesium sulfate. Evaporation of the solvent gave 4.45 g. of the acid, m.p. 104-8°. Recrystallization from acetone-carbon tetrachloride gave m.p. 115-17°. A portion of this acid was treated with diazomethane to give the diester XXIX for VPC comparison. (see below)

By Oxidation of XXVII.

The diamine XXVII (3.6 g.) in 100 ml. of ligroin was added to 200 cc. of water and the mixture cooled to 0°. An alkaline (10% sodium hydroxide) permanganate (5% potassium permanganate) solution was added to the cold mixture with rapid stirring until the mixture acquired a permanent green color (about 1 hr.). The mixture was allowed to stand in the cold for two more hours and sulfur dioxide was passed into the mixture to remove excess oxidizing agent and manganese dioxide. The alkaline solution was extracted with ether and the ether extract set aside.

The aqueous solution was acidified with hydrochloric acid and extracted with ether (800 ml.); the ether extract was washed twice with water and dried over magnesium sulfate. Evaporation of the ether gave 0.690 g. of a low-melting solid. The solid was treated with diazomethane and the resulting oil, XXIX, analyzed by VPC (see below).

Vapor Phase Chromatographic Analysis of XXIX.

All columns were 0.25-in. diameter x 10 ft. in length aluminum columns; helium flow rate and temperature are noted after each column. Injections were made of the diamine oxidation product (as dimethyl esters), and the authentic sample, both separately and simultaneously. The retention time, in minutes, is listed for peaks from the oxidation mixture. The peak that showed the relative increase in size for the simultaneous injection is underlined.

A. 5% SE-30, on Chromosorb W, 60-80 mesh, 53 cc./min., 160°: 2.2, 4.3, 5.0, 6.8, 9.1.

B. Ucon Polar, 133 cc./min., 170°: 0.9, 2.6, 3.4, 4.3, 4.75, 5.6.

C. Craig Polyester succinate, 120 cc./min., 220°: 1.2, 2.9, 5.0.

Preparation of XXIVc from XXVI.

Benzenesulfonyl chloride (1.2 cc., 1.4 g.) was added to a solution of crude XXVI (1.2 g.) in 3 ml. of pyridine and allowed to stand overnight at room temperature. The pyridine solution was poured into cold water and the precipitate filtered off. The precipitate was chromatographed on alumina and eluted with benzene to yield XXIVc, m.p. 103-5°. One crystallization from benzene gave m.p. 105-7°. Mixture m.p. with XXIVc from benzenesulfonyl azide adduct gave m.p. 106-7°.

Preparation of XXIVf from XXVI.

Benzyl chloride (0.8 cc., 0.88 g.) was added dropwise to a mixture of crude XXVI (0.5 g.) and 0.9 g. of sodium bicarbonate in 6 ml. of dioxane and 1 ml. of water. The mixture was refluxed for 2 hours and poured into 10% sodium hydroxide solution. The alkaline solution was extracted with ether and the ether extract was washed with water and dried over magnesium sulfate. Evaporation of the solvent gave an oil which was chromatographed on alumina (and eluted with benzene). The chromatographed oil had an n.m.r. spectrum almost identical to that of the photolysis product of XXVII.

Preparation of Benzoyl Azide.

Benzoyl azide was prepared as previously described[50] with following modifications.

Freshly distilled benzoyl chloride (50 ml., b.p. 193°) in 50 ml. of acetone was added to 60 g. of sodium azide in 100 ml. of water and 50 ml. of acetone over 0.5 hr. at 10°. The suspension was allowed to come to room temperature and stirred overnight. Water was added to the suspension and the mixture extracted with ether. The ether solution was washed with water, three 100 ml. portions of 10% sodium hydroxide solution (the last portion stirred for ½ hr.), and twice with water, and finally dried over magnesium sulfate. Evaporation of the solvent at about 30° under reduced pressure gave 49.7 g. of benzoyl azide as an oil (95%).

Preparation of N-Benzyl-3-azatricyclo(3.2.1.$0^{2,4\text{-exo}}$)octane (XXIVf) by Photolysis.

The triazole XXVII (m.p. 73-4°, 10 g.) was suspended in 400 ml. of hexane by mechanical stirring. The suspension was irradiated with a Hanovia 200-W, type S, u.v. lamp with a quartz well for 15 minutes past the time of complete dissolution of the solid. Evaporation of the solvent gave 8.71 g. of slightly yellow oil whose n.m.r. spectrum showed 75% aziridine and 25% triazoline determined by integration of the H-2,3 protons. The crude product was distilled (92°/0.5 mm.) and redistilled (85°/0.2 mm.) to give 4.695 g. (53%) of colorless (XXIVf) as an oil.

Anal. Calcd. for $C_{14}H_{17}N$: C, 84.37; H, 8.60. Found: C, 84.96; H, 8.6.

N.m.r. (in CS_2) δ 0.63 (H-7a, doublet, J=10 c.p.s.), 1.0-1.2 (four protons), 1.40 (two protons, H-2, H-3), 1.63 (H-7s, doublet, J=9, c.p.s.), 2.25 (two protons, H-1, H-4), 3.19 (two benzylic protons) and 7.23 (five aromatic protons).

Decomposition of 1-Benzyl-3a,4,5,6,7,7a-hexahydro-4,7-methano-1H-benzotriazole (XXVII) with Hydrochloric Acid.

Compound XXVII (9.73 g., 0.0417 moles, m.p. 73-74°) was slurried in 250 ml. of distilled water. Concentrated HCl was slowly added with rapid stirring at room temperature until the solid dissolved (0.5 hr.). The acidic solution was immediately cooled in an ice bath and 20% KOH solution added until the pH was greater than 10. The precipitate first appeared at pH 7 but more precipitate appeared upon further addition of base. The aqueous solution was immediately extracted three times with ether. The ether was washed twice with water and dried overnight over

magnesium sulfate. The solvent was evaporated at reduced pressure to give 8.16 g. of colorless oil (all aziridine would give 8.4 g., all amino alcohol would give 9.15 g.). The oil was chromatographed (TLC, silica gel G, 18 cm., ethyl acetate as mobile phase, (1) = most intense spot). Rf: 0.06(3), 0.14(2), 0.19(?), 0.25(broad, 1), 0.32(3), 0.50(4), 0.68(2).

The crude oil (4.977 g.) was placed on a deactivated alumina column (200 g., basic, act. II, M. Woelm, Eschwege, Germany) and 100-ml. fractions taken. The aziridine, identified by n.m.r. and i.r. comparison with an authentic sample, was eluted in benzene to give 1 g., 20%, as an oil. Amino alcohol B was eluted in 10% ether-benzene (some fractions were contaminated and therefore no yield was calculated. N.m.r. (in $CDCl_3$ containing D_2O) δ 0.85-2.35 (eight protons), 3.78 (H-2 and two benzylic protons), 2.95 (H-7).

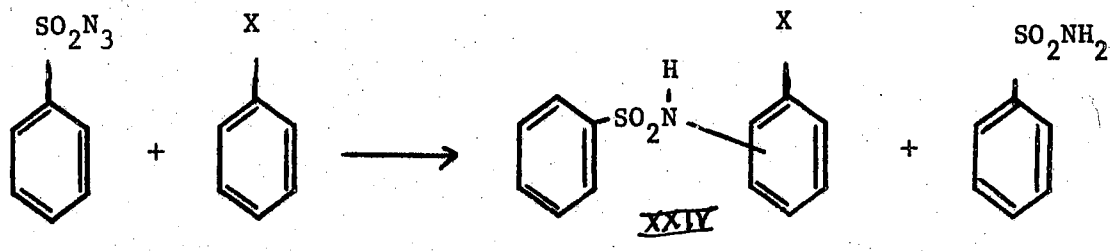
Amino alcohol C was eluted in 50% ether-methanol to give 0.5 g., 10%, as an oil. N.m.r. (in $CDCl_3$) δ 0.82-2.18 (nine protons), 2.72 (one proton, disappears with D_2O), 3.20 (H-7), 3.70 (H-2 and two benzylic protons).

CHAPTER IV

ADDITION OF BENZENESULFONYL AZIDE TO SUBSTITUTED BENZENE DERIVATIVES

A. Historical and Introduction

The thermal decomposition of sulfonyl azides in aromatic solvents was first studied by Curtius and Schmidt[51,52,53] in the 1920's. Later Dermer and Edmison[54] and Heacock and Edmison[55] investigated the ratio of ortho-, meta-, and para- substitution products in a variety of aromatic solvents. Although Heacock and Edmison's results indicated that



XXIV

the isomer ratio in the substitution reaction was like that of homolytic substitution, the relative rate of substitution was less than or equal to that of benzene. Investigation of partial rate factors for ortho-, meta-, and para- substitution products in radical reactions shows that there is a definite tendency for ortho-substitution.[53] In addition, all substrates, ArX, are more reactive than benzene.[53] Heacock and Edmison concluded that the reaction was not a straightforward radical substitution and they proposed an electrophilic diradical intermediate. It has been reported that p-toluenesulfonyl azide does initiate the 1:1 copolymerization of styrene and methyl methacrylate at 60-120° by

a free radical mechanism; however the activity is low.[56]

Heacock and Edmison's isolation of the sulfonanilides (XXXV) was not unambiguous. These workers extracted the sulfonanilides from the reaction mixture with base and there was a possibility that some isomers might be extracted preferentially. In addition, the isomer ratios of the substituted anilides as determined by IR analysis differed somewhat depending on whether they were determined under competitive or non-competitive conditions. A more sensitive analytical method which demanded a minimum amount of work-up was required. Gas chromatography seemed the ideal tool.

The present investigation was undertaken to a) unambiguously determine the ratio of these substitution products, b) note the differences, if any, in the product ratios obtained by pyrolysis and by photolysis of benzenesulfonyl azide in anisole, toluene, chlorobenzene and methyl benzoate.

B. Results and Discussion

Heacock and Edmison's results might be better explained by postulating cyclic intermediates which decompose preferentially to the products. Two basic mechanisms can be proposed for the thermal decomposition of benzenesulfonyl azide. (Figure 5).

In mechanism A, an initially formed triazoline decomposes first to an intermediate aziridine which decomposes to the products. In mechanism B, the benzenesulfonyl azide loses nitrogen to form a diradical which adds to benzene to form directly an intermediate aziridine. Mechanism A cannot be used to explain the photolytic reaction of benzenesulfonyl azide in aromatic solvents.

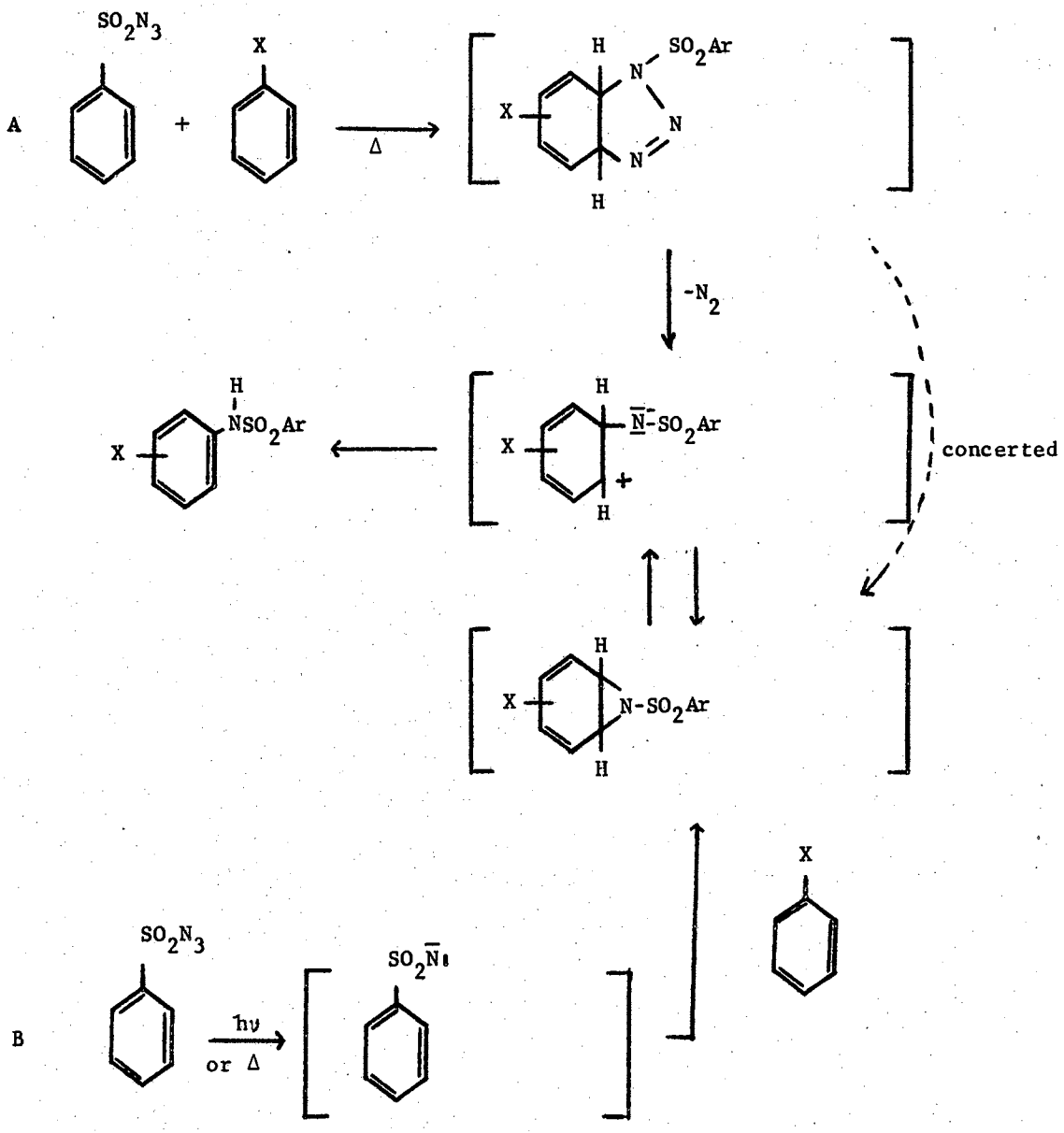


Figure 5. Photolysis and Thermolysis of Benzenesulfonyl Azide.

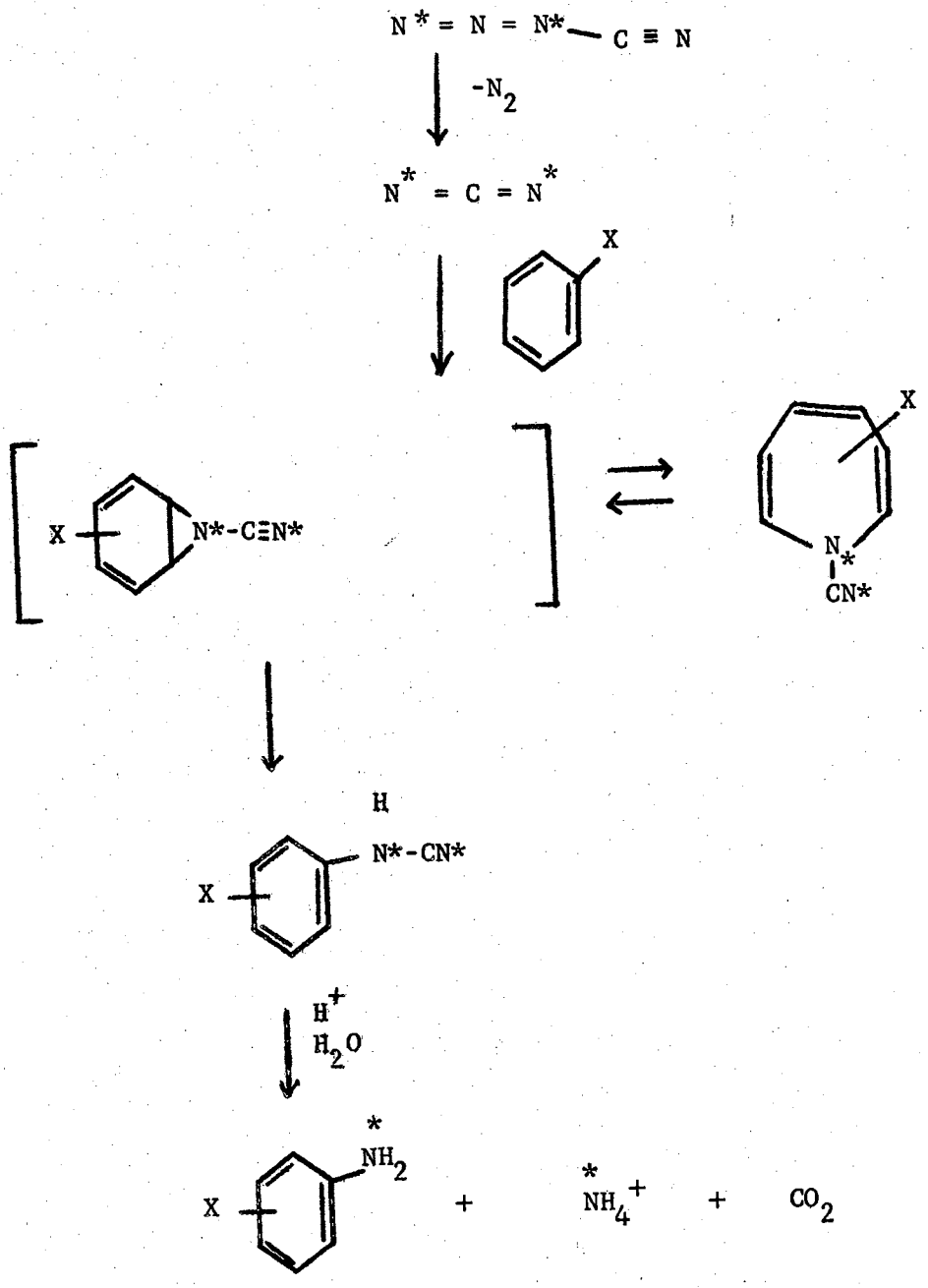


Figure 6. Thermolysis of Cyanogen Azide.

A third variation may also be considered (Figure 6); recently Marsh and Simmons[58] reported that thermolysis of cyanogen azide at 45-60° in aromatic solvents gives N-cyanoazepines, some of which have been shown to rearrange to the ortho- and para- substituted phenylcyanamides, presumably[59] through the aziridine intermediate. These workers used cyanogen azide with the 1 and 3-azido nitrogen atoms labeled with N^{15}. Hydrolysis of the cyanamide gave the corresponding aniline containing only 25% of the original N^{15}. Only scrambling of the nitrogen atoms in the intermediate cyanonitrene can account for this observation since labeled cyanogen azide adds to olefins without scrambling the label.[60]

Recently Abramovitch[61] and co-workers investigated the pyrolysis of methanesulfonyl azide in aromatic solvents in some detail. These workers also propose aziridine intermediates which decompose to substituted anilides. In addition, they have shown that the total amount of methanesulfonamide produced in the reactions cannot be formed by the stepwise abstraction of hydrogen atoms from the substrate. These workers suggest that the methanesulfonamide may arise from the aziridine intermediate decomposing to form the amide and benzyne. Certainly the production of benzyne could account for the abundance of tars formed in these reactions.

The results of this investigation, which are tabulated below along with those of Heacock and Edmison and Abramovitch and co-workers, indicate that in the pyrolysis and photolysis of benzenesulfonyl azide differences are evident which cannot be explained by experimental error. These differences may perhaps be explained by the difference in temperature at which the reactions were run. A change in the ratio of ortho-, meta-, and para- isomers for the competitive and non-competitive runs

occurs in both the thermolysis and photolysis reactions with benzenesulfonyl azide; thus it was also reported to occur in the case of methanesulfonyl azide. This effect is still unexplained.

The tendency for ortho- substitution in the case of toluene and anisole may be attributed to the formation of intermediate C in addition to A and B. That this intermediate may not be ruled out is indicated by the fact that Marsh[58,59] obtained all three possible azepines from cyanogen azide with toluene and chlorobenzene even though the azepines rearranged to only the ortho- and para- substituted cyanamides. Obviously C can only rearrange to the ortho- product because of the large driving force toward aromatization of the ring. The formation of intermediate C also explains the tendency for ortho- substitution in toluene and anisole since, if only A and B are considered, both ortho- and para- positions have a statistically equal chance of forming. The fact that the ortho- to para- ratio is not quite 2 to 1 in the anisole case may be attributed to the fact that C is sterically hindered and would not be expected to form as readily as A and B. It can be shown that for electron-donating groups ring opening of A and B to give meta- products is not as well stabilized by resonance. In the case of C, ring opening to give ortho- products is the only possible pathway. (Figure VII.)

The total rate ratio, ${}^{X}_{H}K$, of benzenesulfonamidation shows that we are dealing with an extremely reactive intermediate. The more reactive an intermediate is, the less it should discriminate between two competing substrates and the closer the value of the total rate ratio should be to unity. This is the situation for the thermolysis of benzenesulfonyl azide in toluene and anisole. However, the reaction is highly selective as to position as well; clearly as in the case of

TABLE VII

TOTAL RATE RATIOS FOR SULFONAMIDATION OF C_6H_5X

Substrate	Total Rate Ratio $\frac{X}{H}K$			
	$C_6H_5SO_2N_3$ [a]	$C_6H_5SO_2N_3$ [b]	$C_6H_5SO_2N_3$ [c]	$CH_3SO_2N_3$ [d]
Toluene	1.16	0.61*	1.00	1.86
Anisole	1.00	2.94	0.91	2.54
Methyl benzoate	0.23	0.79	0.43	----
(Chlorobenzene)	----	----	0.69	0.44

[a] thermolysis, T = 115°

[b] photolysis, T = 20°

[c] Heacock's results[55]

[d] Abramovitch's results[61]

*Calculated by triangulation

TABLE VIII

ISOMER RATIOS FOR SULFONAMIDATION OF C_6H_5X[e]

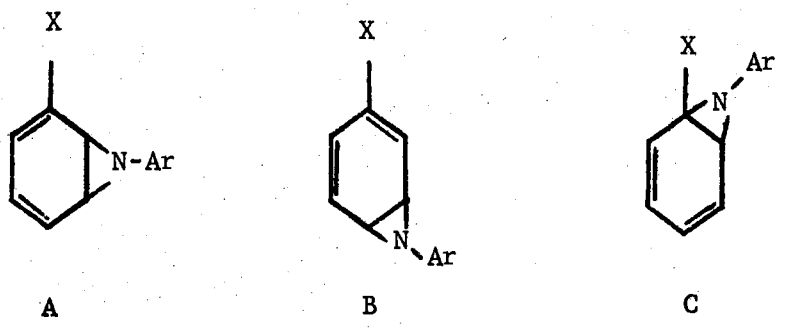
Substrate	Isomer Ratio											
	$C_6H_5SO_2N_3$[a]			$C_6H_5SO_2N_3$[b]			$C_6H_5SO_2N_3$[c]			$CH_3SO_2N_3$[d]		
	o	m	p	o	m	p	o	m	p	o	m	p
Toluene	72	-	28	52	-	48	60.7	1.5	37.4	65.4	2.4	32.2
Anisole	56	-	44	59	-	41	71.5	1.8	26.7	55.5	1.2	43.3
Methyl benzoate	39	7	54	32	34	34	44.3	53.2	2.5	~~57.4~~	~~0.9~~	~~41.7~~
Chlorobenzene	-	-	-	-	-	-	-	-	-	57.4	0.9	41.7

TABLE IX

ISOMER RATIOS FOR COMPETITIVE SULFONAMIDATION OF C_6H_5X

Substrate	Isomer Ratio								
	$C_6H_5SO_2N_3$[a]			$C_6H_5SO_2N_3$[b]			$C_6H_5SO_2N_3$[c]		
	o	m	p	o	m	p	o	m	p
Toluene	66	-	34	82	-	18	61.2	0.5	38.3
Anisole	59	-	41	68	-	32	64	0.0	35.5
Methyl benzoate	35	-	65	40	19	41	38.8	35.5	5.7

[e]Error is ± 2%, detection limits are less than 1%.



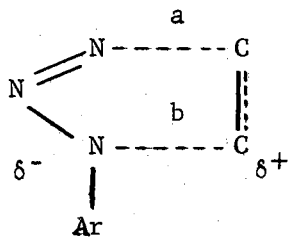
A B C

methanesulfonamidation, the rate-determining-step is not the product-determining-step. In fact, in the case of the thermolysis in toluene and anisole, it may be that the rate-determining-step is the decomposition of the azide rather than addition to the substrate.

It is unfortunate that Abramovitch did not choose methyl benzoate as the substrate, and that we could not separate the products from the chlorobenzene reaction to complete the picture. However the limited results that can be compared indicate that in the thermolyses, benzenesulfonylnitrene is more reactive than methanesulfonylnitrene since the total rate ratios of the benzenesulfonyl azide cases are closer to unity.

The fact that little or no meta- isomer was observed by any workers in the toluene and anisole cases indicates there must be an aziridine or closely related symmetrical intermediate formed in the course of the reaction even if a triazoline intermediate is involved. A particular triazoline can only open to form one unique sulfonamide if no symmetrical intermediate is involved.

Scheiner[62] suggests a one-step transition state for the addition of azides to olefins where bond a is more completely formed than bond b in the transition state.



On this basis, the transition state leading to the triazoline where the partial positive charge is best stabilized would be favored. In the toluene and anisole cases the favored transition states would lead to ortho- and para- substituted products and little or no meta- products would be expected.

It is tempting to speculate that azepine formation may occur in the thermolysis and photolysis of benzenesulfonyl azide. Although the temperature of thermolysis would probably preclude isolation of any azepines[58,59], it may be possible to isolate any azepines formed in the photolysis since the reaction is run at 20°. In the present study azepines would not be detected due to the higher temperature of the work-up and the high column temperature in the gas chromatography. It is probably dangerous at this stage to draw any far-reaching conclusions from the results obtained in this study because both in the thermolysis and photolysis experiments, large amounts of tars of unknown origin and composition were formed.

C. Experimental

All solvents were dried by refluxing over anhydrous barium oxide and distilling prior to use. Solutions were made up to contain 0.0050 mole fraction of benzenesulfonyl azide. In the case of competitive reactions, benzene and the other substrate were each 0.500 mole fraction

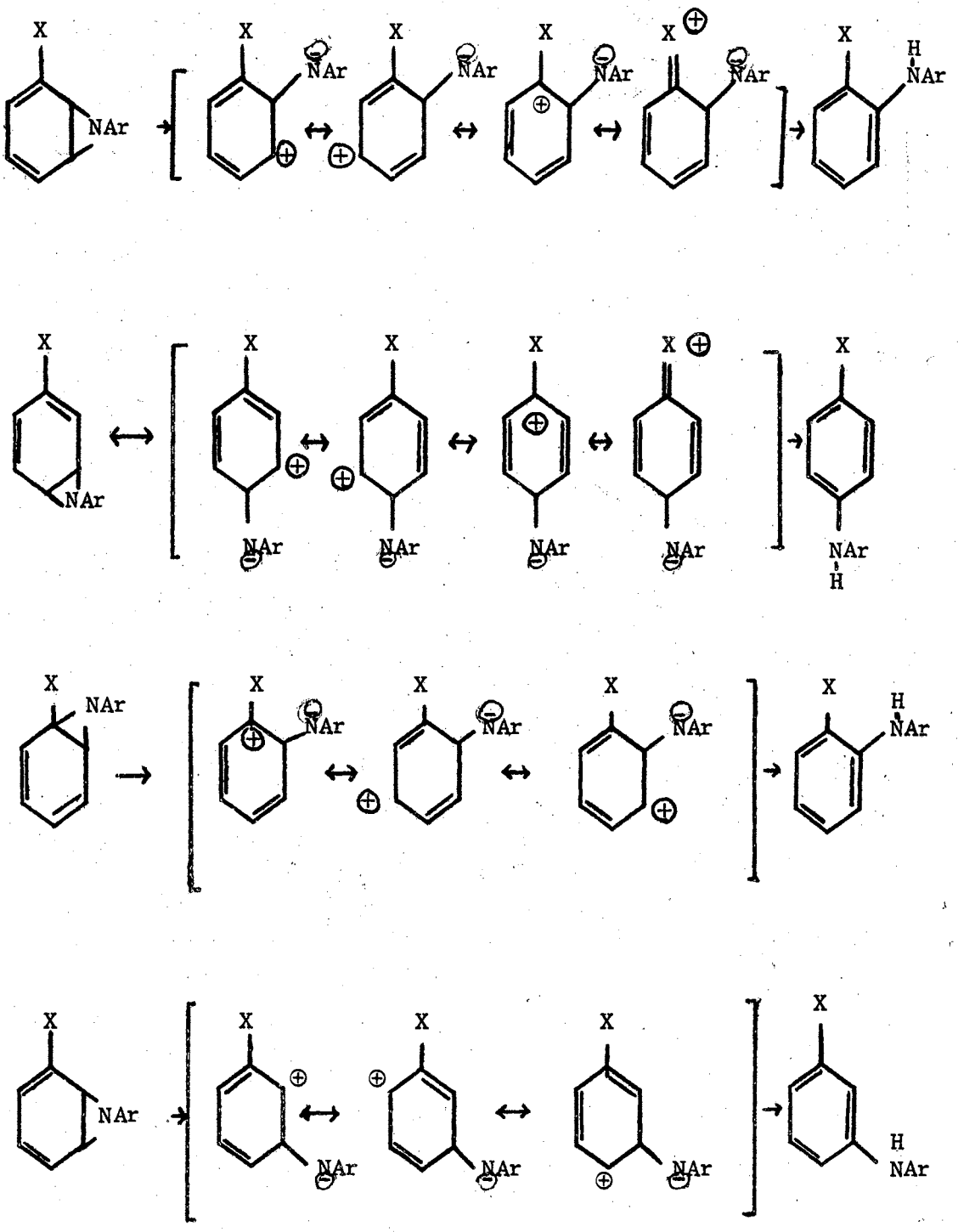


Figure 7. Proposed Modes of Decomposition of Sulfonamidation Intermediates.

and benzenesulfonyl azide was 0.0050 mole fraction. In each case the solutions used in photolysis and pyrolysis were from the same mixture. All photolyses were run at approximately 20° C. for 5 hours using a Hanovia 200-W. U.V. lamp. All pyrolyses were run (by A. C. Oehlschlager) in a sealed tube at 115° C. for 2 days. The reaction mixtures were concentrated under vacuum on a steam bath. The residues were dissolved in acetone and filtered from a black precipitate. The precipitate was washed with acetone (the sulfonanilides are easily soluble in acetone) and the washings combined with the filtrate. The mixtures were analyzed using a 5-6% SE-52 on Chrom/G (60/80 mesh) in a 13' x ¼" aluminum column on an Aerograph A-700 Autoprep with thermal detector. Samples were injected directly on the column by using an 8" syringe needle since it was found that repeated injections of the reaction mixtures clogged the injector port. Authentic samples of the various benzenesulfonanilides were obtained through the courtesy of Professor O. C. Dermer.

Calculation of the relative rate of substitution, ${}^{X}_{H}K$, with respect to benzene may be made using the following equation[63].

$$ {}^{X}_{H}K = \frac{k_X}{k_H} = \frac{\log\left[1 - \frac{Z_o R}{X_o(1+R)}\right]}{\log\left[1 - \frac{Z_o}{H_o(1+R)}\right]} $$

Where k_H and k_X are specific rate constants for benzene and a substituted benzene respectively and H_o and X_o are initial concentrations of benzene and the competing substrate and Z_o is the initial concentration of the compound which is being competed for, and R is the ratio of products.

The molar ratio of the products is not in general directly the ratio of the reaction velocities of the respective substitutions, since the molar composition of the mixture of solvents changes during the course of the reaction. However this error is minimized by using a large excess of the two competing reactants so as to make their concentrations essentially constant. The product ratio then gives $\frac{X}{H}K$ directly and obviates the use of the above equation.[64]

Sample Calculations

The following is a set of sample calculations with the thermolysis of benzenesulfonyl azide with the anisole case used as an example. The values obtained in these calculations do not correspond exactly to the values in the tables since the latter are averages of several runs.

Compound	Relative area from gas chromatography
o-benzenesulfonanisidide	49
p-benzenesulfonanisidide	27
benzenesulfonanilide	26
benzenesulfonamide	688
	790

$$\% \text{ benzenesulfonamide} = \frac{688}{790} \times 100 = 87$$

isomer ratio

$$\% \text{ o-benzenesulfonanisidide} = \frac{49}{49 + 27} \times 100 = 64.5$$

$$\% \text{ p-benzenesulfonanisidide} = \frac{27}{49 + 27} \times 100 = 35.5$$

total rate ratio $\frac{X}{H}K$

$$\frac{X}{H}K = \frac{49 + 27}{26} = 2.92$$

Gas Chromatographic Analysis of the Products from the Benzenesulfonamidation of Toluene.

The following method of quantitative analysis of incompletely resolved peaks was worked out in detail by T. H. Austin[65]. A tracing of a typical chromatogram is pictured and the symbols which are given are used in the description. The area corresponding to benzenesulfonanilide was taken as (A+B+C)-B. The area of this peak as with all others was measured with an Ostwald planimeter. The area of o-benzenesulfonotoluidide was taken as (B+C+C)-C.

The following is a tabulation of retention times for components of benzenesulfonamidation of the various substrates.

TABLE X

GAS CHROMATOGRAPHY OF SULFONAMIDATION PRODUCTS

Substrate	Retention Time (Min.)					Flow, ml/min	Column*** T_c, °C	Injector T_i, °C
			Anilides					
	BSA*	BSAN**	o	m	p			
Toluene	7	23.5	26	-	30	50	250	320
Anisole	3.3	6.9	9.0	-	11	93	290	320
Methyl benzoate	5.5	8.1	13.3	15.3	17.2	75	282	330

*benzenesulfonamide

**benzenesulfonanilide

***An Aerograph A-700 Autoprep with thermal detector was used with a 5-6% SE-52 on Chrom G (60/80 mesh), 13' x ¼" aluminum column.

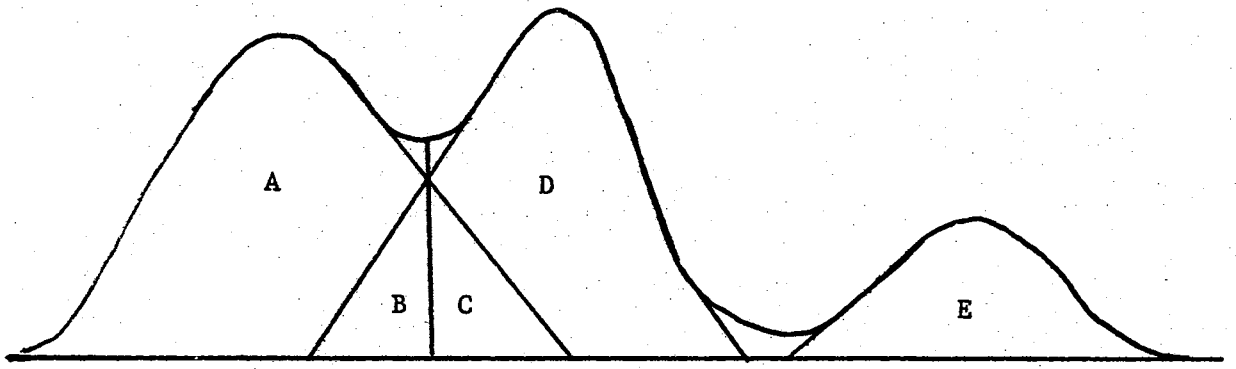


Figure 8. Typical Chromatogram of Benzenesulfonamidation of Toluene where the Peaks were not Completely Resolved.

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