

Anal. Calcd for $C_{14}H_{15}N_2O_6P \cdot H_2O$: C, 47.20; H, 4.81; N, 7.86. Found: C, 46.79; H, 4.40; N, 7.82.

Inner Salt of 1-(2'-*n*-Amyl hydrogen phosphoxy-5'-nitrobenzyl)-pyridinium Hydroxide (7b). In a similar manner, 7b was obtained from *n*-amyl 2-chloromethyl-4-nitrophenyl hydrogen phosphate (4b) and aqueous pyridine in 41% yield as white prisms: mp 139–140° (acetone-ethanol); R_f 0.78 (isopropyl alcohol-concentrated ammonium hydroxide-water, 7:1:2, v/v); $\lambda_{max}^{H_2O}$ 261 m μ (log ϵ 3.95), 267.5 (3.95), and 292 (3.96).

Anal. Calcd for $C_{17}H_{21}N_2O_6P \cdot H_2O$: C, 51.26; H, 5.82; N, 7.03. Found: C, 51.73; H, 5.90; N, 7.13.

Alkyl Dihydrogen Phosphate (8). General Procedure. A solution of 0.01 mol of alkyl 2-chloromethyl-4-nitrophenyl hydrogen phosphate (4) in a mixture of pyridine (4.0 ml, 0.05 mol) and water (5.4 ml, 0.3 mol) was kept at room temperature for 2 days and then heated at 80° for 8 hr. To the reaction mixture, 20 ml of absolute ethanol was added, and the mixture was stirred at room temperature for several minutes. A yellow precipitate, 1-(2'-hydroxy-5'-nitrobenzyl)pyridinium chloride (hydrogen chloride salt of 9), was filtered and washed with two 20-ml portions of absolute ethanol. From the combined alcoholic filtrate and washings, the corresponding alkyl dihydrogen phosphates (8) were isolated as monoanilinium salts by means of either method A or method B. *n*-Hexadecyl 8c, benzyl 8f, and phenyl dihydrogen phosphate 8g were obtained according to method A and the other derivatives listed in Table II were isolated by method B.

Method A. The combined alcoholic filtrate and washings were evaporated to dryness under reduced pressure and the residue was dissolved in 50 ml of dry acetone. A small amount of insoluble yellow crystals was removed by filtration and then 2 ml of aniline was added to the filtrate. The mixture was stored in a refrigerator overnight to give monoanilinium alkyl dihydrogen phosphate (8) as white crystals.

Method B. To the combined alcoholic filtrate and washings was added 40 ml of water and the resulting solution was passed through a column (12 \times 200 mm) of Amberlite IR 120 resin (H^+ form). The column was washed with 50% ethanol until the washings were no longer acid to litmus. The combined eluent and washings were evaporated to dryness under reduced pressure. The pale yellow syrupy residue was dissolved in 10 ml of 95%

ethanol (in the case of $R = n$ -amyl, 10 ml of dry ether was used) and then 2 ml of aniline was added to the solution. The mixture was allowed to stand in a refrigerator overnight to give the monoanilinium salt of 8 as white crystals.

1-(2'-Hydroxy-5'-nitrobenzyl)pyridinium Chloride (Hydrogen Chloride Salt of 9). Two grams (0.0107 mol) of 2-chloromethyl-4-nitrophenol (1) was dissolved in 20 ml of dry pyridine. An exothermic reaction occurred and a yellow precipitate soon appeared. The reaction mixture was stirred at room temperature for 2 hr. Then the precipitate was collected by filtration and washed with ethanol to give 2.7 g (95%) of the HCl salt of 9. Repeated recrystallization by means of hot water afforded pale yellow prisms for an analytical sample: mp >250°; R_f 0.71 (isopropyl alcohol-concentrated ammonium hydroxide-water, 7:1:2, v/v); $\lambda_{max}^{H_2O}$ 260 m μ (log ϵ 3.85), 266.5 (shoulder), and 3.16 (3.93).

Anal. Calcd for $C_{12}H_{11}ClN_2O_3$: C, 54.04; H, 4.16; N, 10.51. Found: C, 54.16; H, 4.45; N, 10.62.

Inner Salt of 1-(2'-Ethyl hydrogen phosphoxy-5'-nitrobenzyl)-triethylammonium Hydroxide (10). A solution of 3.0 g (0.01 mol) of ethyl 2-chloromethyl-4-nitrophenyl hydrogen phosphate (4a) in 20 ml of triethylamine was warmed at 50–55° for 6 hr. After removal of excess triethylamine by evaporation, the resulting oily residue was dissolved in 50 ml of dry acetone and the solution was allowed to stand at room temperature about for 2 hr. The triethylammonium chloride which separated was removed by filtration. The filtrate was kept standing in a refrigerator overnight to afford 2.6 g (72%) of the crude 10: mp 177–178° dec. It was purified by passing through a column of Amberlite IRC 50 (H^+ form) as mentioned in the above experiment (preparation of 7a). Repeated recrystallization by means of acetone containing a trace of water afforded white cubes for an analytical sample: mp 175–176° dec; R_f 0.67 (isopropyl alcohol-concentrated ammonium hydroxide-water, 7:1:2); $\lambda_{max}^{H_2O}$ 288 m μ (log ϵ 3.95).

Anal. Calcd for $C_{15}H_{23}N_2O_6P$: C, 50.00; H, 6.99; N, 7.77. Found: C, 49.77; H, 7.04; N, 7.75.

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Metal-Ammonia Reduction. IV.¹ Single-Stage Reduction of Polycyclic Aromatic Hydrocarbons

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Abstract: Reduction by lithium dissolved in liquid ammonia of a series of representative polycyclic aromatic hydrocarbons (naphthalene, dibenz[*a,h*]anthracene, 9-alkyl- and 9,10-dialkylanthracene, 7-methyl- and 7,12-dimethylbenz[*a*]anthracene, 3-methylcholanthrene, phenanthrene, and pyrene) was selectively directed to the dihydro stage. Product structure accorded, in general, with predictions based upon molecular orbital calculations of the positions of highest electron density in the corresponding anionic intermediates. Reduction of 9,10-dialkylanthracene proceeded stereospecifically when the alkyl function was ethyl, benzyl, or *n*-butyl to furnish *trans*-9,10-dialkyl-9,10-dihydroanthracene; when the alkyl group was methyl, *cis* and *trans* diastereomers were formed in equal proportion. The *cis*-diethyl, dibenzyl, and di-*n*-butyl isomers were independently synthesized, and differences in the chemical reactivity of the *cis* and *trans* diastereomers were related to the conformational properties of the hydrocarbons and their anionic derivatives; preferential axial attack during alkylation or protonation is proposed.

Reduction of polycyclic aromatic hydrocarbons, despite a long history of investigation,² remains one of the least predictable and controllable reactions of the

polynuclear hydrocarbons. Not only do chemical reduction and catalytic hydrogenation frequently lead to different products, but variations of either method often influence both the extent of reduction and the distribution of isomeric hydroaromatic products. Much of this complexity is a consequence of secondary processes which include isomerization of double bonds, dispro-

(1) Part III: R. G. Harvey and K. Urberg, *J. Org. Chem.*, **33**, 2206 (1968).

(2) E. Clar, "Polycyclic Hydrocarbons," Vol. I and II, Academic Press, New York, N. Y., 1964.

portionation of radical-anionic intermediates, facile further reduction, and surface phenomena.³

Efficient stepwise reduction of anthracene to hexahydroanthracene⁴ and benz[*a*]anthracene to dodecahydrobenz[*a*]anthracene¹ by lithium dissolved in liquid ammonia was described in parts I–III of this series. Reactions conducted under appropriately controlled conditions proceeded smoothly with high specificity to provide products whose structures were in accord with qualitative prediction based on consideration of the relative stabilities of the intermediate dianions or radical anions.

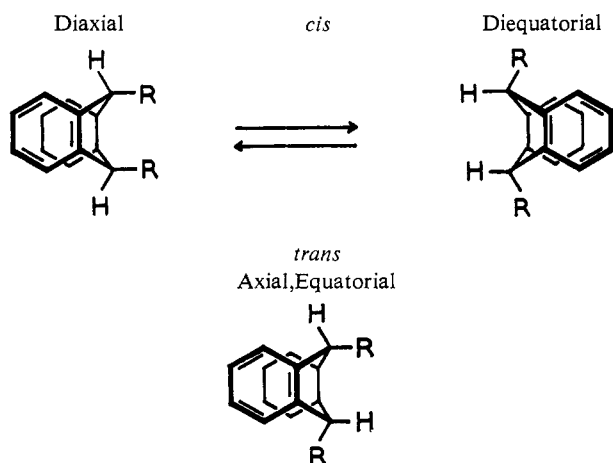
The present paper reports extension of the method to the single-stage reduction of a series of representative polycyclic aromatic hydrocarbons (naphthacene, dibenz[*a,h*]anthracene, 9-alkyl- and 9,10-dialkylanthracene, 7-methyl- and 7,12-dimethylbenz[*a*]anthracene, 3-methylcholanthrene, phenanthrene, and pyrene). Evidence is presented concerning the stereochemistry and detailed mechanism of reduction.

Results

The presence of alkyl substituents in the *meso* positions of anthracene and benz[*a*]anthracene failed to deactivate the central ring sufficiently to favor reduction elsewhere in the molecule. Thus, 9-methyl- and 9-ethylanthracene as well as 9,10-dimethyl-, diethyl-, dibenzyl-, and di-*n*-butylanthracene as well as 9-ethyl-10-methylanthracene underwent reduction by lithium in ammonia to furnish the corresponding 9-monoalkyl- and 9,10-dialkyl-9,10-dihydroanthracene derivatives in good yield. Also, 7-methylbenz[*a*]anthracene, 7,12-dimethylbenz[*a*]anthracene, and 3-methylcholanthrene were similarly transformed to the analogous 7,12-dihydrobenz[*a*]anthracene derivatives.

As pointed out by Beckett and Mulley,⁵ the benzylic positions of 9,10-dihydroanthracene contain two conformationally distinct types of bonds, designated as quasiaxial (*a'*) and quasiequatorial (*e'*), which are interconvertible *via* "boat-to-boat" ring inversion. As a consequence, 9,10-dialkyldihydroanthracenes may exist as *cis* (diaxial \rightleftharpoons diequatorial) or *trans* (axial,equatorial \rightleftharpoons equatorial,axial) diastereomers (Chart I). Deter-

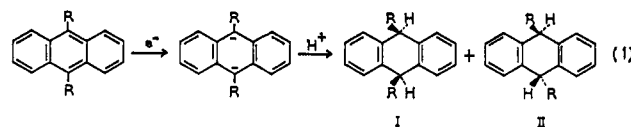
Chart I. 9,10-Dialkyl-9,10-dihydroanthracene



- (3) G. J. Hoijtink, *Rec. Trav. Chim. Pays-Bas*, **76**, 885 (1957).
 (4) R. G. Harvey, *J. Org. Chem.*, **32**, 238 (1967); R. G. Harvey and K. Urberg, *ibid.*, **33**, 2570 (1968).
 (5) A. H. Beckett and B. A. Mulley, *J. Chem. Soc.*, 4159 (1955).

mination of the ratio of isomers from reduction of the fully aromatic precursors would provide insight into the essentially unknown stereochemistry of metal-ammonia reduction of hydrocarbons.⁶

Gas chromatographic analysis of the product of lithium-ammonia reduction of 9,10-dimethylanthracene revealed *cis*- and *trans*-9,10-dimethyl-9,10-dihydroanthracenes (I, II; R = CH₃), in approximately equal proportion (eq 1). I (R = CH₃), the less-soluble, higher



melting (mp 130–131°) isomer, exhibited a shortened retention time (8.6 min) on the gas chromatography column⁷ than II (R = CH₃) (mp 101°; retention time 10.6 min). The structures of I and II (R = CH₃) were previously established by stereospecific synthesis from the diacids of known configuration;^{8,9} these assignments were counter to those suggested earlier by Badger, *et al.*¹⁰ The *cis* isomer underwent smooth catalytic dehydrogenation (91% conversion in 6 hr) with 10% palladium on charcoal in refluxing diglyme, whereas the *trans* isomer proved resistant (11% conversion in the same period), a difference which further supports the assigned structures. Equilibration of pure I (R = CH₃) with *n*-butyllithium in tetrahydrofuran for 6 hr furnished essentially the same ratio of isomers (53% *cis*) as lithium-ammonia reduction of dimethylanthracene (52% *cis*). On the other hand, reduction with sodium in refluxing alcohol provided a mixture richer in the *cis* isomer (78% *cis*).

The nmr spectrum of I (R = CH₃) revealed a quartet in the benzylic region at τ 5.92 (2 H, *J* = 7.5 Hz) and a doublet for the methyl protons at τ 8.47 (6 H, *J* = 7.5 Hz); the spectrum of II (R = CH₃) displayed analogous peaks at τ 5.94 and 8.38 (*J* = 7.5 Hz). The expected magnetic nonequivalence of groups in the *a'* and *e'* orientation was not evident in these spectra. This is interpreted as due to ring inversion sufficiently rapid on the nmr time scale (*i.e.*, $\lesssim 50$ sec⁻¹) to result in a "time-average" resonance signal. It is significant, however, that the methyl doublet of the *cis* isomer appears at higher field than that of the *trans* isomer. Since the chemical shift of *trans*-methyl must represent an equal *a'*-*e'* contribution, the shift of *cis*-methyl to higher field is indicative of unequal residence time in the two possible conformations. Axial methyl groups lie in the positive shielding region over the aryl rings and should therefore appear at higher field than the equatorial methyl groups which lie in the plane of the ring where they should be deshielded by the ring current and shifted to lower field.¹¹ Support for this predicted relationship is found in the report by Barclay and Chapman¹² of the nmr spectrum of 9,10-dimethyl-9,10-ethano-9,10-dihydroanthracene, a molecule in which geometrical re-

- (6) H. Smith, "Organic Reactions in Liquid Ammonia," Vol. 1, Part 2, John Wiley & Sons, Inc., New York, N. Y., 1963.

(7) A 6-ft column of 10% SE-30 on Chromosorb W at 150° was employed.

- (8) A. H. Beckett and R. G. Lingard, *J. Chem. Soc.*, 2409 (1959).

- (9) L. M. Jackman and J. W. Lown, *ibid.*, 3776 (1962).

- (10) G. M. Badger, M. L. Jones, and R. S. Pearce, *ibid.*, 1700 (1950).

- (11) C. E. Johnson and F. A. Bovey, *J. Chem. Phys.*, **29**, 1012 (1958).

- (12) L. R. C. Barclay and R. A. Chapman, *Can. J. Chem.*, **43**, 1754 (1965).

strictions permit the methyl groups to occupy only equatorial positions. The methyl protons were found at lower field (τ 8.04) than the methylene protons (τ 8.34). Also, Lansbury, Bieron, and Lacher¹³ found in the 7,12-dihydropleiadene series that the axial methoxymethyl group appears at τ 6.65 whereas its equatorial counterpart appears at τ 6.38. Thus it is reasonable to suggest that the diaxial form of I ($R = CH_3$) predominates over its diequatorial counterpart. It is hoped that more direct evidence on this question will be provided by low-temperature nmr studies currently in progress.

Reduction of 9,10-diethyl-, dibenzyl-, di-*n*-butyl-, and 9-ethyl-10-methylanthracene proceeded, in contrast to that of 9,10-dimethylanthracene, with a high degree of stereospecificity to furnish a single isomer in 90–95% yields. These were assigned the *trans* structure for reasons discussed in the following paragraphs.

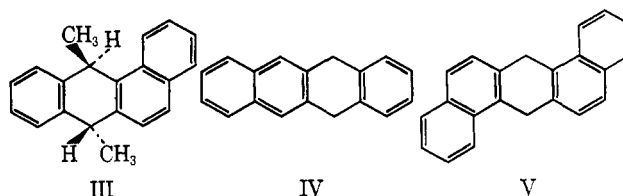
The corresponding *cis*-9,10-diethyl-, dibenzyl-, and di-*n*-butyl isomers (I; $R = C_2H_5$, $C_6H_5CH_2$, $n-C_4H_9$), required for comparison, were synthesized *via* alkylation of 9,10-dihydroanthracene with *n*-butyllithium and the corresponding alkyl bromide in liquid ammonia.¹⁴ *cis*-9-Ethyl-10-methyl-9,10-dihydroanthracene was synthesized *via* addition of ethyllithium to anthracene in tetrahydrofuran¹⁵ and alkylation of the resulting monoethylanion with methyl bromide.^{14,16}

The *cis*-diethyl like the *cis*-dimethyl was the less-soluble, higher melting isomer (mp 58–59° *vs.* 44–45°); however, I ($R = C_2H_5$) exhibited slightly longer retention time than II ($R = C_2H_5$) (16.2 *vs.* 15.3 min) on the gas chromatographic column.⁷ More significantly, the rate of catalytic dehydrogenation of I ($R = C_2H_5$) with 10% palladium on charcoal in refluxing diglyme greatly exceeded (90% conversion in 12 hr) that of the *trans* isomer (2% conversion in the same period), in agreement with expectation. *cis*-9-Ethyl-10-methyl-9,10-dihydroanthracene was, like the *cis*-dimethyl and the *cis*-diethyl analogs, the higher melting, more readily dehydrogenated isomer (mp 103–104° *vs.* oil). The dibenzyl hydrocarbons (I, II; $R = C_6H_5CH_2$) were similarly related with regard to melting point (112 *vs.* 94°). The di-*n*-butyl compounds (I, II; $R = n-C_4H_9$) were both oils. Dehydrogenation was not investigated.

The nmr spectrum of I ($R = C_2H_5$) contained a triplet in the benzylic region (2 H, $J = 7$ Hz) at τ 6.23, an apparent quintet for the methylene protons (4 H, $J = 7$ Hz) at τ 8.22, and a methyl triplet (6 H, $J = 7$ Hz) at τ 8.95. Similarly, the nmr spectrum of II ($R = C_2H_5$) exhibited analogous peaks with similar splitting patterns at τ 6.02, 7.92, and 9.20, respectively. The appearance of *cis*-methylene upfield from *trans*-methylene is in agreement with the analogous relationship observed for *cis*- and *trans*-methyl signals of I and II ($R = CH_3$).⁹ The nmr spectra of both the *cis*- and *trans*-9-ethyl-10-methyl isomers were consistent with those of I and II ($R = CH_3$ and C_2H_5). The spectra of I and II ($R = C_6H_5CH_2$) followed the same pattern in that *cis*-methylene appeared upfield (τ 7.02) from

trans-methylene (τ 6.78). Finally, the spectra of I and II ($R = n-C_4H_9$) were, unfortunately, too complex in the methylene region for satisfactory analysis.

Although, *cis* and *trans* pairs of enantiomers are conceivable for 7,12-dimethyl-7,12-dihydrobenz[*a*]anthracene, the product of reduction (mp 89–91.5°) appeared from its nmr spectrum to consist of a single diastereomer; this was tentatively assigned the *trans* structure (III) on the basis of the observed preference for *trans* reduction in the foregoing examples, and by comparison with the authentic *cis* isomer, synthesis of which will be described in the following paper in this series.



Transformation of naphthacene and dibenz[*a,h*]anthracene with lithium (2.5 equiv) in liquid ammonia under standard conditions proceeded smoothly to furnish 5,12-dihydronaphthacene (IV) and 7,14-dihydrodibenz[*a,h*]anthracene (V), respectively, as the sole products.¹⁷ The nmr spectra of IV and V exhibited, in agreement with the assigned structures, four benzylic protons (singlets at τ 6.25 and 5.72 for IV and V, respectively) in addition to the appropriate number of aromatic protons. The melting point of V (227.3–228.3°) was somewhat higher than that reported by either Lijinsky¹⁹ (222.5–223.5°) or Bachmann²⁰ (218.5–219.5°), and considerably higher than that reported¹⁹ for the 5,6-dihydro isomer (194–195°). The latter structure was conclusively ruled out by the close correspondence between the ultraviolet spectrum of the product and the published spectrum of V which, in turn, differed from that of 5,6-dihydrodibenz[*a,h*]anthracene.¹⁹

Thus, naphthacene and dibenz[*a,h*]anthracene undergo reduction, like anthracene and benz[*a*]anthracene, exclusively in the *meso* region. This confirms Streitwieser's prediction, based upon molecular orbital calculations of the positions of highest electron density,²¹ and suggests that single-stage reduction of other linearly fused hydrocarbons, or *acenes*,² may be expected to take place analogously.

Birch reduction of phenanthrene reportedly²² furnishes 1,2,3,4,4a,9,10,10a-octahydrophenanthrene (VI) as the sole product. It was suggested²² that, since analogous reaction of 9,10-dihydrophenanthrene also provides VI, initial reduction occurs at the 9,10 bond. Hückel and Bretschneider²³ reported isolation of a small quantity of 9,10-dihydrophenanthrene from treatment of phenanthrene with two atomic proportions of sodium or one of calcium in ammonia. Under our standard conditions, lithium–ammonia reduction of phenanthrene was largely limited to a single stage, fur-

(13) P. T. Lansbury, J. F. Bieron, and A. J. Lacher, *J. Amer. Chem. Soc.*, **88**, 1482 (1966).

(14) R. G. Harvey and C. C. Davis, *J. Org. Chem.*, in press.

(15) D. Nicholls and M. Szwarc, *J. Amer. Chem. Soc.*, **88**, 5757 (1966).

(16) H. Zieger and D. Schaeffer, Abstracts of the 155th National Meeting of the American Chemical Society, San Francisco, Calif., April 1968, P155.

(17) No additional products were revealed by nmr or by tlc of the crude product on trinitrobenzene-impregnated silica gel.¹⁸

(18) R. G. Harvey and M. Halonen, *J. Chromatog.*, **25**, 294 (1966).

(19) W. Lijinsky, *J. Org. Chem.*, **26**, 3230 (1961).

(20) W. E. Bachmann, *ibid.*, **1**, 347 (1936).

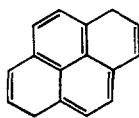
(21) A. Streitwieser, Jr., "Molecular Orbital Theory for Organic Chemists," John Wiley & Sons, Inc., New York, N. Y., 1961, p 425.

(22) S. Mejer, *Bull. Acad. Polon. Sci. Chim.*, **9**, 773 (1961).

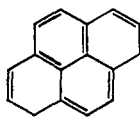
(23) W. Hückel and H. Bretschneider, *Ann.*, **540**, 157 (1939).

nishing 9,10-dihydrophenanthrene (65% by glpc analysis). The nmr spectrum of the crude product displayed, in addition to the expected aromatic (8 H) and benzylic (4 H) protons (singlet at τ 6.80), minor peaks in the vinylic and allylic regions which gradually disappeared upon standing. Evidence for 2,4a,9,10-tetrahydrophenanthrene as the structure of this unstable secondary product will be published separately as part of a detailed study of reduction in the phenanthrene series.²⁴

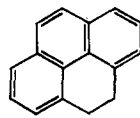
Pyrene, upon treatment under standard conditions, with 2.5 equiv of lithium in liquid ammonia, provided only recovered pyrene and a small proportion of an extensively reduced product. A complex series of color changes was observed during quenching with alcohol. A similar reaction (10 min instead of 1 hr) quenched rapidly with water proceeded more cleanly to furnish in addition to recovered pyrene a substance (~50%) exhibiting vinylic (4 H), allylic (4 H), and aromatic (4 H) protons in the nmr spectrum. Either the 1,6-dihydro- or the 3,6-dihdropyrene structure (VII or VIII) is compatible with the integrated proton ratios. The



VII



VIII



IX

compound underwent facile isomerization to the stable 4,5-dihdropyrene (IX) (detected by nmr spectroscopy) during column chromatography on silica gel as well as during gas chromatography on various columns at 150–175°. The 1,6- (or, 3,6-) dihydropyrene structure was claimed earlier by Neunhoeffer, Woggon, and Dähne,²⁵ on the basis of ultraviolet spectral and chemical evidence, for the dihydropyrene formed upon simultaneous addition of sodium and alcohol to pyrene in liquid ammonia. In view of the herein demonstrated instability of 1,6- (or 3,6-) dihydropyrene relative to the 4,5 isomer, it appears likely that similar rearrangement may also complicate any attempt to characterize this substance by chemical means. Nmr, therefore, provides the least equivocal basis for structural assignment.

Discussion

The efficiency of lithium dissolved in liquid ammonia as a reagent for selective single-stage reduction of fused-ring aromatic molecules is clear from the foregoing results. Reactions of benz[a]anthracene,²⁶ phenanthrene,^{27,28} chrysene,²⁹ etc., which require days in refluxing ether for maximum yield, are complete in minutes at –33° in liquid ammonia.³⁰ These differences undoubtedly reflect both the enhanced solubility of alkali metals in polar media and a shift in equilibria to favor radical-anionic and dianionic intermediates.⁶

Aside from the obvious synthetic utility of the method, the significant feature is its specificity with regard to

both the position and the stereochemistry of reduction. According to molecular orbital theory, the site of reduction should correspond to the region of highest electron density in the anionic intermediates; the calculated values are independent of whether electrons are added individually or in pairs.²¹

The observed reduction of anthracene and its benzo and dibenzo analogs (benz[a]anthracene, naphthacene, and dibenz[a,h]anthracene) in the *meso* region is in accord with predictions based upon molecular orbital calculations by Streitwieser.²¹ Analogous transformation of *meso*-substituted alkyl derivatives of anthracene and benz[a]anthracene in the central ring is somewhat more surprising. Alkyl groups should, *a priori*, be expected to adversely influence formation of negatively charged intermediates at the position substituted. This effect may be further augmented where such groups impose stereochemical limitations on carbanion geometry by their large size or by being part of a strained ring system. Nevertheless, even 9,10-di-*n*-butylantracene and 3-methylcholanthrene underwent exclusive reduction in the *meso* region; no products of alternate ring transformation were detected in any case. Unfortunately, calculated predictions are unavailable for these substituted polycyclic aromatic systems. The dihydro derivatives obtained from phenanthrene and pyrene were also in accord with theoretical prediction.²¹

The remarkable stereospecificity of reduction of 9,10-diethylantracene, as well as the apparent absence of steric preference in reduction of 9,10-dimethylantracene, is explicable as follows. Stabilization of the 9,10-dianion by orbital overlap with the adjacent aromatic rings would require substituents at C-9,10 to occupy pseudoequatorial positions (eq 2). Whether as a consequence the ring system tends to become planar because of increased sp^2 character at positions 9 and 10 is a moot point; it should be kept in mind that the number of electrons involved exceeds the requirement for a normal sp^2 hybrid. Initial protonation at C-9 would be expected to occur from an axial direction for maximum overlap to furnish a monoanion (Ia) free to invert. Where the substituent at C-9 is sufficiently small so that *peri* interaction is negligible, the second protonation may occur with equal facility in either conformation of the monoanion (Ia or IIa). This is presumably the case when this group is methyl, since *cis* and *trans* products are formed equally. However, the greater bulk of the ethyl substituent forces it to favor the conformation offering minimum steric interaction. This is presumed to be the inverted form (IIa) in which the ethyl group is axial.³¹ Protonation of this leads to the experimentally observed *trans* isomer (eq 3).

Steric control was also evident during alkylation of dialkyldihydroanthracene; the *trans*-diethyl isomer (II; R = C₂H₅) was smoothly transformed to 9,9,10-triethyl-9,10-dihydroanthracene (97%) upon treatment with *n*-butyllithium and ethyl bromide, whereas the *cis* isomer (I; R = C₂H₅) proved relatively resistant (18%). The *trans*-diethyl isomer failed to equilibrate in the presence of *n*-butyllithium, but did furnish 9,10-diethylantracene (30%) and 9-ethylantracene (12%) as well as recovered II (R = C₂H₅). The *cis*-diethyl isomer

(24) P. Rabideau and R. G. Harvey, unpublished results.

(25) O. Neunhoeffer and H. Woggon, *Ann.*, **600**, 34 (1956); O. Neunhoeffer and S. Dähne, *ibid.*, **612**, 98 (1958).

(26) W. E. Bachmann, *J. Org. Chem.*, **1**, 347 (1936).

(27) W. Schlenk and E. Bergmann, *Ann.*, **463**, 84 (1928).

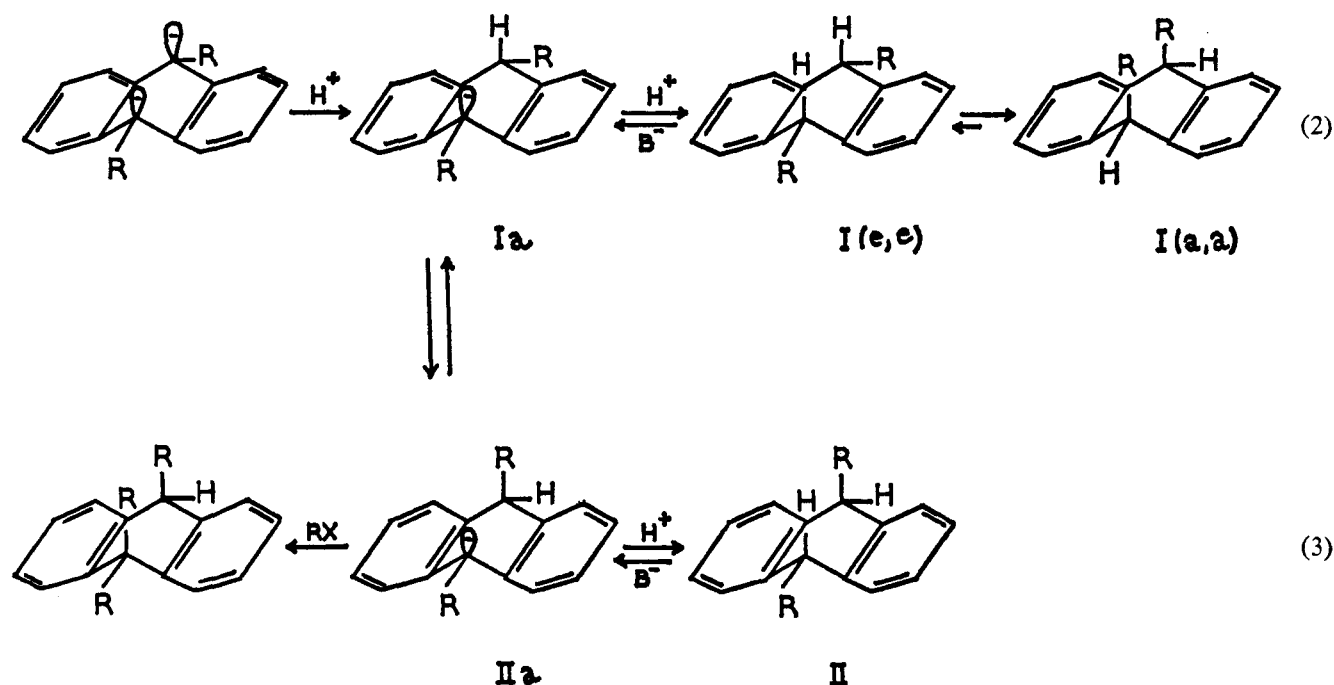
(28) A. Jeanes and R. Adams, *J. Amer. Chem. Soc.*, **59**, 2608 (1937).

(29) S. E. Hunt and A. S. Lindsey, *J. Chem. Soc.*, 2227 (1958).

(30) Metal-ammonia reduction of chrysene is under current investigation.

(31) Molecular models of the space-filling type (e.g., Courtault) indicate ethyl, isopropyl, and *n*-butyl groups in the quasiaxial orientation to be subject to considerably greater nonbonded interaction with *peri* hydrogens than are their quasiaxial counterparts.

Chart II



equilibrated to modest extent, to provide an estimated 10% yield of II ($\text{R} = \text{C}_2\text{H}_5$).

These findings are consistent with the proposed mechanism (Chart II). Consider first the *trans* diastereomer. Proton abstraction evidently occurs with facility during alkylation, so that failure to observe equilibration indicates that proton return on IIa must take place from the same direction (presumably axial) as proton abstraction from II. The secondary products (diethyl- and monoethylanthracene) may be rationalized as arising *via* elimination of hydride ion and ethyl anion, respectively, from Ia and/or IIa. The reluctance of the *cis* diastereomer to undergo either alkylation or equilibration is most simply interpreted as a consequence of existence primarily in a conformation unfavorable for proton removal; presumably this is the diaxial configuration in which both benzylic protons are equatorial (eq 2). It is notable, however, that some equilibration of the *cis*, but not of the *trans*, isomers did take place; this agrees with expectation that equilibrium between Ia and IIa favors the latter.

Although 7-methylbenz[*a*]anthracene, 7,12-dimethylbenz[*a*]anthracene, and 3-methylcholanthrene are among the most potent carcinogenic substances known,³² their reduction paralleled the analogous transformation of benz[*a*]anthracene and other structurally related, but biologically inactive, hydrocarbon molecules. Reductive behavior, like other forms of chemical reactivity^{33,34} (with the possible exception of osmium tetroxide oxidation), thus fails to correlate with carcinogenic activity.

Experimental Section

Physical Data. Melting points were taken on a Leitz Kofler hot-stage microscope and are corrected. Proton nmr spectra were obtained on a Varian Model A-60 spectrometer; chemical shifts

are reported relative to tetramethylsilane in deuteriochloroform. Gas chromatographic analyses were performed on a F & M, Model 500, chromatograph; larger scale separations (100–500 mg) were achieved on a Hewlett-Packard, Model 775, preparative chromatograph.

Materials and Methods. The polycyclic aromatic hydrocarbons were obtained from commercial sources, recrystallized from absolute alcohol, and dried *in vacuo*. If chromatography on trinitrobenzene-silica gel¹⁸ revealed impurities after crystallization, the hydrocarbons were further purified by passage through a column of activated silica gel. This procedure is essential for hydrocarbons known to form transannular peroxides (e.g., 7,12-dimethylbenz[*a*]anthracene). Silica gel for column chromatography (Davison, Grade 950, mesh 60–200) was activated by heating overnight at 100°. Tetrahydrofuran (THF) was distilled from LiAlH_4 and stored over CaH_2 under nitrogen. Lithium wire (Lithium Corp. of America) was wiped free of oil and washed with hexane immediately before use.

All reductions in ammonia were carried out employing essentially standard conditions^{1,4} described for reduction of 9-methylanthracene, unless indicated otherwise. Precautions for the exclusion of moisture and atmospheric oxygen were scrupulously followed; ammonia was distilled into the reaction vessel through a column of barium oxide (10–20 mesh); all reductions were carried out under helium for reasons stated earlier.¹ Thin layer chromatograms on silica gel impregnated with trinitrobenzene¹⁸ were developed in benzene-heptane, 1:4.

9-Methyl-9,10-dihydroanthracene. To a stirred solution of lithium (872 mg, 12.5 g-atoms) dissolved in liquid ammonia (150 ml) was added 9-methylanthracene (961 mg, 5 mmol) in 75 ml of THF. The solution was maintained at reflux for 1 hr, then the deep red color was discharged by addition of alcohol (5 ml), followed by water (10 ml). Evaporation of the ammonia followed by partition of the residue between ether and water led to recovery of an oily solid; by tlc it appeared to consist of a single product (R_f 0.35) free of 9-methylanthracene (R_f 0.18). Recrystallization from methanol provided white needles (680 mg) melting at 59–60° (lit.³⁵ 58.8–59.8°); nmr 437 (s, 8, aryl), 240 (q, 1, CHCH_3 , $J = 7.5$ Hz), 228 (d, 1, CH_2 , $J = 18$ Hz), 250 (d, 1, CH_2 , $J = 18$ Hz), and 81 cps (d, 3, CH_3 , $J = 7.5$ Hz).

9-Ethyl-9,10-dihydroanthracene. Repetition of the foregoing experiment with 9-ethylanthracene provided an oily solid purified by chromatography on silica gel to give 9-ethyl-9,10-dihydroanthracene (87%) whose nmr spectrum corresponded closely to that described and interpreted by Nicholls and Szwarc.¹⁵

(32) C. B. Huggins, J. Pataki, and R. G. Harvey, *Proc. Natl. Acad. Sci. U. S.*, **58**, 2253 (1967).

(33) J. C. Arcos and M. Arcos, *Progr. Drug Res.*, **4**, 407 (1962).

(34) R. G. Harvey and M. Halonen, *Cancer Res.*, **28**, 2183 (1968).

(35) D. D. Phillips and J. Cason, *J. Amer. Chem. Soc.*, **74**, 2934 (1952).

cis- and *trans*-9,10-Dimethyl-9,10-dihydroanthracenes (I and II; R = CH₃). Products from lithium-ammonia reduction of 9,10-dimethylantracene were separated by glpc⁷ into the *cis* (52%) and the *trans* (48%) isomers of 9,10-dimethyl-9,10-dihydroanthracene. The former, the less soluble of the two isomers, crystallized from alcohol and melted at 130–131° (lit.⁸ 130–131°). Although II could not be completely freed of I by crystallization, the pure isomer was readily obtained by preparative glpc.

A more efficient route to the *cis* diastereomer was *via* treatment of a solution of 9,10-dimethylantracene (1 g) in refluxing ethanol (150 ml) with sodium (9 g) added in portions over a period of 30 min. Glpc analysis indicated 9,10-dimethyl-9,10-dihydroanthracene as the sole product with the *cis-trans* isomers present in the ratio 4:1.

9,10-Dimethylantracene. A gentle stream of nitrogen was bubbled through a refluxing solution of I (R = CH₃) (200 mg) in diethylene glycol dimethyl ether (25 ml) in the presence of 10% palladium on charcoal (40 mg). Samples were removed every hour by syringe and analyzed by glpc.⁷ In 6 hr, reaction attained virtual completion; the product consisted of 9-methyl-9,10-dihydroanthracene (2%), I (R = CH₃) (3%), II (R = CH₃) (1%), unknown (4%), and 9,10-dimethylantracene (91%). Analogous transformation of II (R = CH₃) achieved only 11% conversion in 6 hr and 24% conversion in 12 hr.

9,10-Dimethylantracene was also obtained *via* treatment of I (R = CH₃) (1.04 g) with 2,3-dichloro-5,6-dicyanobenzoquinone (DDQ) (1.14 g) in refluxing benzene for 16 hr. Chromatography on a column of silica gel followed by recrystallization from ethanol furnished the pure hydrocarbon (65%) melting at 180–181° (lit.¹⁰ 180°); nmr 182 cps (s, 6, CH₃).

trans-9,10-Diethyl-9,10-dihydroanthracene (II; R = C₂H₅). The sole product from lithium-ammonia reduction of 9,10-diethylantracene was II (R = C₂H₅) (100%); mp 44–45°. The absence of the *cis* diastereomer was indicated by glpc⁷ (retention time 15.3 min *vs.* 16.2 min for the *cis* isomer) and by nmr (spectra discussed in the body of the paper).

Anal. Calcd for C₁₈H₂₀: C, 91.45; H, 8.53. Found: C, 91.23; H, 8.60.

9,10-Diethylantracene. Catalytic dehydrogenation under the same conditions employed for the methyl homologs converted 90% of I (R = C₂H₅), but only 2% of II (R = C₂H₅), to the corresponding diethylantracene in a 12-hr period. Recrystallization from ethanol furnished 9,10-diethylantracene: mp 146–147° (lit.¹⁶ 147–148°); nmr 219 (q, 4, CH₂, *J* = 7.5 Hz) and 87 cps (t, 6, CH₃, *J* = 7.5 Hz).

Anal. Calcd for C₁₈H₁₈: C, 92.26; H, 7.74. Found: C, 92.19; H, 7.74.

Aromatization of I (R = C₂H₅) with DDQ in refluxing benzene under the same conditions employed for the methyl homolog provided a mixture of products containing 9,10-diethylantracene and recovered II (R = C₂H₅), 65 and 13%, respectively, by glpc.⁷

trans-9,10-Dibenzyl-9,10-dihydroanthracene (II; R = C₆H₅CH₂). The sole product from lithium-ammonia reduction of 9,10-dibenzylantracene was II (R = C₆H₅CH₂): mp 94°; nmr 423, 403 (m, 18, aryl), 247 (t, 2, CH, *J* = 6), and 199 cps (d, 4, CH₂, *J* = 6).

Anal. Calcd for C₂₈H₂₄: C, 93.29; H, 6.72. Found: C, 92.20; H, 6.95.

9,10-Dibenzylantracene. Due to the thermal instability of I (R = C₆H₅CH₂),¹⁴ catalytic dehydrogenation was not attempted. Instead, a solution of I (R = C₆H₅CH₂) (5.4 g, 15 mmol) in THF (60 ml) was treated with a solution (1.6 *M*) of *n*-butyllithium (30 mmol) in hexane for 2 hr, then oxidized by addition of solid iodine (excess). Recrystallization of the crude product from benzene (charcoal) provided 9,10-dibenzylantracene (0.98 g), mp 243–245°; nmr 495 (m, 4, aryl), 445 (m, 4, aryl), 428 (s, 10, aryl), and 302 cps (s, 4, CH₂).

Anal. Calcd for C₂₈H₂₂: C, 93.81; H, 6.19. Found: C, 93.42; H, 6.36.

cis-9-Ethyl-10-methyl-9,10-dihydroanthracene. A solution of ethyllithium (54 mmol) in benzene was added over 15 min to a solution of anthracene (4.44 g, 25 mmol) in THF (125 ml) at 0°. The resulting solution was decolorized by methyl bromide after 40 min, stirred an additional 10 min, then worked up according to standard procedure to afford a white solid product (5.63 g), 94% pure by glpc. Recrystallization from ethanol provided the pure

compound (3.53 g, 64%): mp 103–104°; nmr 59 (t, 3, CH₂CH₃, *J* = 7 Hz), 91 (d, 3, CH₃, *J* = 7 Hz), 106 (qu, 2, CH₂, *J* = 7 Hz), 227 (t, 1, CHCH₂, *J* = 7 Hz), 243 (q, 1, CHCH₃, *J* = 7 Hz), and 432 cps (s, 8, aryl).

Anal. Calcd for C₁₇H₁₈: C, 91.84; H, 8.16. Found: C, 91.87; H, 8.15.

trans-9-Ethyl-10-methyl-9,10-dihydroanthracene. Lithium-ammonia reduction of 9-ethyl-10-methylantracene furnished an oil identified as the *trans* isomer; nmr 53 (t, 3, CH₂CH₃, *J* = 7 Hz), 106 (d, 3, CH₃, *J* = 7 Hz), 231 (t, 1, CHCH₂, *J* = 7 Hz), 240 (q, 1, CHCH₃, *J* = 7 Hz), and 453 cps (m, 8, aryl).

Anal. Calcd for C₁₇H₁₈: C, 91.84; H, 8.16. Found: C, 91.90; H, 8.21.

9-Ethyl-10-methylantracene. Catalytic dehydrogenation of *cis*-9-ethyl-10-methyl-9,10-dihydroanthracene under conditions similar (except reaction time increased to 24 hr) to those employed for dehydrogenation of the *cis*-dimethyl homolog afforded pale yellow crystals of the parent aromatic hydrocarbon, melting at 143–144° (lit.²⁷ 145.5°); nmr 87 (t, 3, CH₂CH₃, *J* = 7.5 Hz), 186 (s, 3, CH₃), and 219 cps (q, 2, CH₂, *J* = 7.5 Hz).

Anal. Calcd for C₁₇H₁₆: C, 92.68; H, 7.31. Found: C, 92.71; H, 7.41.

trans-7,12-Dimethyl-7,12-dihydrobenz[*a*]anthracene (III). Lithium metal (140 mg) was added to a solution of 7,12-dimethylbenz[*a*]anthracene (1.28 g, 5 mmol) in THF (100 ml) and liquid ammonia (250 ml), and the resulting olive-green solution was stirred at reflux for 2 hr. The oily product was chromatographed on silica gel, then dissolved in hot ethanol and added to an equal weight of 2,4,7-trinitrofluorenone (TNF) in benzene-ethanol. Upon concentration and cooling a crystalline TNF complex formed. Chromatography on silica gel furnished crude III (786 mg, mp 84–88°), recrystallization of which from methanol provided the analytical sample, melting at 89–91.5° (an isomer of III of unknown stereochemistry was reported³⁸ to melt at 90–91°); nmr 87 (d, 3, CH₃, *J* = 7 Hz), 103 (d, 3, CH₃, *J* = 7 Hz), 243 (q, 1, CH, *J* = 7 Hz), and 291 cps (q, 1, CH, *J* = 7 Hz).

Anal. Calcd for C₂₀H₁₈: C, 92.98; H, 7.02. Found: C, 92.61; H, 7.09.

7-Methyl-7,12-dihydrobenz[*a*]anthracene. Reduction of 7-methylbenz[*a*]anthracene (5 mmol) in the presence of ferric chloride (40 mg) provided the 7,12-dihydro derivative (80%) free of further reduction products (formed in the absence of iron salts), but contaminated with unreacted 7-methylbenz[*a*]anthracene (20%). 7-Methyl-7,12-dihydrobenz[*a*]anthracene exhibited nmr 253 (q, 1, CHCH₃, *J* = 7 Hz), 272 (d, 1, CH₂, *J* = 19 Hz), 245 (d, 1, CH₂, *J* = 19 Hz), and 82 cps (d, 3, CH₃, *J* = 7 Hz) in addition to the appropriate number of protons in the aryl region.

Anal. Calcd for C₁₉H₁₆: C, 93.40; H, 6.60. Found: C, 93.62; H, 6.48.

3-Methyl-6,12b-dihydrocholanthrene. As in the previous reaction, ferric chloride (40 mg) inhibited reduction beyond the dihydro stage (as determined by nmr). Recrystallization from ethanol provided the *meso*-dihydro derivative (81%), melting at 140.2–140.8° (lit.^{20,39} 136–137°, 138–139°); nmr 135 cps (s, 3, CH₃); the uv spectrum corresponded closely to that reported by Fieser and Hershsberg.³⁹

5,12-Dihydronaphthacene. The sole product (by tlc and nmr) of reduction of naphthacene was 5,12-dihydronaphthacene. Recrystallization from ethanol provided white needles (80%) melting at 216.6–217° (lit.⁴⁰ 205–207°); nmr 225 cps (s, 4, CH₂).

Anal. Calcd for C₁₈H₁₄: C, 93.87; H, 6.13. Found: C, 93.68; H, 6.36.

7,14-Dihydrodibenz[*a,h*]anthracene. The crude product (free of dibenz[*a,h*]anthracene by tlc) was recrystallized from benzene-ethanol to furnish white plates (92%) melting at 227.3–228.3° (lit.^{19,20} 222.5–223.5° and 218.5–219.5°); nmr 257 cps (s, 4, CH₂).

Anal. Calcd for C₂₂H₁₆: C, 94.25; H, 5.75. Found: C, 94.02; H, 5.60.

9,10-Dihydrophenanthrene. Reduction of phenanthrene and a number of its derivatives has been examined in detail and will be reported separately. Reaction under essentially standard conditions in the presence of ferric chloride (lithium added last) furnished 9,10-dihydrophenanthrene (65%). The latter was identified by its

(37) R. Gerdil and E. Lucken, *Helv. Chem. Acta*, **44**, 1966 (1961).

(38) B. M. Mikhailov, *Izv. Akad. Nauk SSSR, Ser. Khim.*, 619 (1946).

(39) L. F. Fieser and E. B. Hershsberg, *J. Amer. Chem. Soc.*, **60**, 940 (1938).

(40) B. M. Mikhailov and A. D. Chinaeva, *Sb. Statei Obshch. Khim., Akad. Nauk SSSR*, **1**, 626 (1963); *Chem. Abstr.*, **49**, 993 (1955).

(36) B. M. Mikhailov and A. N. Blokhina, *Izv. Akad. Nauk SSSR, Ser. Khim.*, 164 (1949); *Chem. Abstr.*, **44**, 2962 (1950).

retention time (3.15 min) on a 6-ft 5% DEGS column at 175°, by its R_f value on TNF-impregnated silica gel,¹⁸ and by its nmr spectrum (four benzylic protons at τ 7.20), all identical with those of an authentic sample.

1,6- (or 3,6-) Dihydropyrene. The reduction of pyrene with lithium in ammonia-THF was quenched after 1 hr as rapidly as possible with water, and the product precipitated by addition of excess water. Tlc on silica gel in cyclohexane indicated the presence of only a single component (R_f 0.11) in addition to unreacted pyrene (R_f 0.28). Gas chromatography on a 6-ft 5% DEGS on Chromosorb W column at 175° similarly exhibited one product (14.6 min) and pyrene (24.2 min). The nmr spectrum of the mixture was consistent with the presence of the predicted 1,6- (or 3,6-) dihydropyrene structure (see Discussion). However, decomposition (primarily isomerization to 4,5-dihydropyrene) took place on both the gas chromatographic column and a column of silica gel at room temperature, preventing isolation of the 1,6- (or 3,6-) dihydro isomer. The identity of 4,5-dihydropyrene was demonstrated by correspondence of the nmr spectrum (notably, the presence of a singlet for four benzylic protons at τ 6.80) and retention time on the glpc column identical with that of the authentic compound.⁴¹

(41) E. A. Coulson, *J. Chem. Soc.*, 1298 (1937).

9,9,10-Triethyl-9,10-dihydroanthracene. To a solution of II ($R = C_2H_5$) (1.18 g, 5 mmol) in THF (50 ml) and ammonia (150 ml) was added a solution of ethyllithium in benzene (5 mmol). After 15 min the red color was discharged by the addition of excess ethyl bromide. Glpc analysis of the oil (1.06 g) obtained upon work-up indicated II ($R = C_2H_5$) (3%) and the 9,9,10-triethyl compound (97%). The assigned structure was confirmed by nmr spectroscopy: 10 (t, 3, CH_3 , $J = 7$ Hz), 38 (t, 3, CH_3 , $J = 7$ Hz), 46 (t, 3, CH_3 , $J = 7$ Hz), 113 (m, 2, CH_2CH), 117 (q, 2, CH_2 , $J = 7$ Hz), 124 (q, 2, CH_2 , $J = 7$ Hz), 240 (t, 1, CH , $J = 6$ Hz), and 436 cps (m, 8, aryl).

Anal. Calcd for $C_{20}H_{24}$: C, 90.85; H, 9.15. Found: C, 91.28; H, 8.84.

Attempted ethylation of I ($R = C_2H_5$) under similar conditions provided the triethyl compound (18%) and unreacted starting material (82%).

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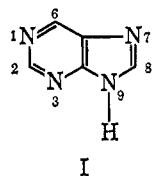
NH-Proton Exchange of Purine in Aqueous Solution. Effect of Molecular Complexing on Reaction Rate¹

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Abstract: The effect of molecular complex formation on the kinetics of NH-proton exchange of purine was studied by the nmr method in aqueous solution at pH 4.2–7.0. At low purine concentrations, the following fast reactions were characterized by kinetic analysis: (a) proton transfer with second-order kinetics involving the reactants purine, water, and purinate ion; rate constant $k_2 = 1.0 \times 10^8 \text{ sec}^{-1} M^{-1}$ at 20°; (b) reaction of purine with hydrogen ion: rate constant $k_{H^+} = 2.4 \times 10^{10} \text{ sec}^{-1} M^{-1}$ at 20°. At 0.85 M , where the average degree of association of purine is 2.01, the rate constants are nearly the same: $k_2 = 1.0 \times 10^8$ and $k_{H^+} = 3.1 \times 10^{10} \text{ sec}^{-1} M^{-1}$ at 20°. The high value of k_{H^+} implies that purinium ion can undergo very rapid proton exchange at N-9, perhaps by a mechanism of reversible ionization. The purine NH–water OH proton chemical shift was found to be 12.4 ppm at 20°.

We wish to report nmr measurements of the kinetics of proton exchange between purine (PuH) and water in the presence of 0.1 N NaCl. It



has been shown by a variety of methods that purine molecules associate in aqueous solution to form molecular stacks,^{2–5} and we were interested in the effect

of that association on proton exchange. Our results may also be pertinent to current work on the mechanism of proton exchange between nucleic acids and water,⁶ by providing data for a simple model system.

Our measurements were made in the pH range 4.2–7.0, in which purine exists largely in the form of the uncharged species, PuH. The measured proton exchange is between NH sites of PuH and OH sites of water. The purine concentration ranged from 0.036 to 0.85 F .

Osmotic data for purine and its derivatives are consistent with the model that association takes place to an infinite degree, and that the equilibrium constants for consecutive association steps are all equal.^{2,5} Let $[PuH]$ denote the formal purine concentration, let $[X_1]$ and $[X_k]$ denote molar concentrations of purine monomer and k -mer, and let K denote the stepwise association constant (eq 1), which by hypothesis is independent of k . For purine in water, $K = 2.4$ at 20° and ΔH°

(1) Work supported by the Petroleum Research Fund of the American Chemical Society. Grateful acknowledgment is made to the donors of that fund.

(2) P. O. P. Ts'o, I. S. Melvin, and A. C. Olsen, *J. Am. Chem. Soc.*, **85**, 1289 (1963).

(3) S. I. Chan, M. P. Schweizer, P. O. P. Ts'o, and G. K. Helmkamp, *ibid.*, **86**, 4182 (1964).

(4) S. J. Gill, M. Downing, and G. F. Sheats, *Biochemistry*, **6**, 272 (1967); E. L. Farquhar, M. Downing, and S. J. Gill, *ibid.*, **7**, 1224 (1968).

(5) G. K. Helmkamp and N. S. Kondo, *Biochim. Biophys. Acta*, **157**, 242 (1968); **145**, 27 (1967).

(6) See, for example, M. P. Printz and P. H. von Hippel, *Proc. Natl. Acad. Sci. U. S. A.*, **53**, 363 (1965); S. W. Englander and J. J. Englander, *ibid.*, **53**, 370 (1965).