removing the acetic anhydride, the residue was taken up in 100 ml. of ether and washed twice with 50-ml. portions of 6% sodium bicarbonate solution followed by distilled water. The ethereal layer was dried over sodium sulfate and taken down to dryness. Four recrystallizations from acetone–petroleum ether afforded 165 mg. of the lactone acetate V, m.p. 128.5–130°, $[\alpha]^{25}D$ −55°, $\lambda_{max}^{CHCl_3}$ 5.80 μ.

Anal. Calcd. for $C_{15}H_{22}O_4$: C, 67.64; H, 8.33. Found: C, 68.08; H, 8.51.

***anti-trans*-1-Keto-8β-methyl-4,5-(4-keto-1,2,3 4-tetrahydrobenzo)-hydrindane (VII).**—*Pseudomonas testosteroni* (ATCC 11996) was grown in 1.6 l. (four 2-l. erlenmeyer flasks) of Difco nutrient broth on a rotary shaker. After 24 hr. of incubation, 288 mg. of a racemic mixture of VI in 6.4 ml. of dimethylformamide was distributed equally among the four flasks. After 24 hr., the culture broth was acidified with 6 *N* hydrochloric acid (20 ml.) and extracted with 1.5 l. of chloroform in 3 portions. The combined chloroform extract was dried over sodium sulfate and taken down to dryness to give 1.23 g. of residue. An aliquot of the chloroform extract was chromatographed on paper and developed for 3 hr. in a toluene–propylene glycol system; a new product appeared with an R_f value 0.73 as viewed under the ultraviolet scanner.[18] This product was isolated by dissolving 1.23 g. of residue in chloroform and streaked across 10 sheets of Whatman No. 1 paper (8 × 18.5 in.) and developed in the toluene–propylene glycol system for 4 hr. The faster moving band was cut, eluted with methanol–chloroform (1:1) and concentrated to dryness. Crystallization of the residue from acetone–petroleum ether afforded 125 mg. of VII, m.p. 137–138°, $[\alpha]^{26}D$ +92°; $\lambda_{max}^{CHCl_3}$ 5.75, 6.01 and 6.17 μ; λ_{max}^{alc} 239 mμ (ε 14,500)

Anal. Calcd. for $C_{14}H_{18}O_2$: C, 77.03; H, 8.31. Found: C, 77.23; H, 8.59.

Ozonolysis of (+)-*anti-trans*-1-Keto-8β-methyl-4,5-(4-keto-1,2,3,4-tetrabenzo)-hydrindane (VII).—The diketo-tetrabenzohydrindane VII (90 mg.) was dissolved in 5 ml. of ethyl acetate and 4 ml. of acetic acid and ozonized (1.5 molar equivalents) at −10 to −15°. The resulting light yellow solution was diluted with 10 ml. of water and 1 ml. of hydrogen peroxide and allowed to stand in an ice-box for 24 hr. The colorless solution was diluted with 40 ml. of ether and the organic layer washed three times with 10-ml. portions of water to remove the acetic acid. The ether solution was extracted with three 20-ml. portions of 6% sodium bicarbonate solution; the basic extracts were acidified with 6 *N* hydrochloric acid and extracted with chloroform. The combined chloroform extracts were dried over sodium sulfate and concentrated to give an oil. The oil was taken up in chloroform and chromatographed over 10 g. of silicic acid. Elution with methanol–chloroform (2:98) yielded 69 mg. of crude crystals. Two recrystallizations from acetone–petroleum ether gave 49 mg. of the acid II, m.p. 109–110°, identical in all respects (m.m.p., infrared spectra and mixed paper chromatography) with a sample obtained by *N. restrictus* fermentation.

Microbiological Oxidation of 9,10-*seco*-Phenol (I) into 3aα-H-4aα-[3′-Propionic acid]-7aβ-methyl-hexahydro-1,5-indanedione (II).—*Nocarcia restrictus* No. 545, *Bacterium cyclo-oxydans* (A.T.C.C. 12673, *Pseudomonas testosteroni* (A.T.C.C. 11996) and *Mycobacterium rhodochrous*[19] were used. Each organism was cultivated in 50 ml. of Difco nutrient broth (250-ml. erlenmeyer flasks) for 24-hr. on a rotary shaker at 25°, 12 mg. of 9,10-*seco*-phenol (I) in 0.2 ml. of dimethylformamide was added to each flask and samples were taken every 6 hr. after steroid addition for analysis by paper chromatography. After 18 hr., all organisms produced a new acidic product with an R_f value of 0.07 in the toluene–propylene glycol system and 0.27 in the butanol–ammonium system.[20] Mixed paper chromatography with authentic samples of II in these systems showed no resolution.[21]

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(21) NOTE ADDED IN PROOF.—Since the manuscript was accepted, the enol lactone III (20 mg.) has also been obtained from the 120 mg. of oily residue (R_f 0.76), by rechromatographing it on the same chromatographic column using cyclohexane–toluene (9:1) as the mobile phase. The stereochemistry of the 5-hydroxyl group in the dihydroxy acid IV is still under scrutiny.

[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY, CLARK UNIVERSITY, WORCESTER, MASS., AND THE WORCESTER FOUNDATION FOR EXPERIMENTAL BIOLOGY, SHREWSBURY, MASS.]

The Anthrasteroid Rearrangement. XI. The Conversion of $\Delta^{5,7,9}$-Anthrapregnatrien-20-one to 4′,10-Dimethyl-1,2-benzanthracene by a Model of a Biochemical Route[1,2]

BY WILLIAM R. NES AND DWAIN L. FORD[3]

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$\Delta^{5,7,9}$-Anthrapregnatrien-20-one after 17α-hydroxylation was converted *via* the D-homosteroid rearrangement and dehydrogenation to 4′,10-dimethyl-1,2-benzanthracene. The latter was identical with a sample prepared by total synthesis. The structure and spectroscopic properties of several intermediates and by-products are discussed. The major conversions leading from the anthrasteroid to the benzanthracene parallel known types of biochemical reactions, and this or a related sequence is suggested as a possible route for the biological formation of a carcinogen.

Within a few years just before and after 1930, Clar[4] devised a simple synthesis of 1,2,5,6-dibenzanthracene, and Kennaway[5,6] was able to show it to be a potent carcinogen; this was the first defined compound found to have cancer-producing properties. In the same period, Cook, Hewett and Hieger[7] discovered that 3,4-benzpyrene was the carcinogen which had the fluorescence spectrum of coal tar, and the structure of the steroids was finally elucidated due to the efforts of Rosenheim and King[8] and of Wieland and Dane.[9] These rather remarkable discoveries prompted Cook and Kennaway[10] to propose, in 1932, that steroids might be converted *in vivo* to a polycyclic aromatic hydrocarbon, and in the following year Cook and Haslewood[11] and Wieland and Dane[12] actually achieved the non-biological conversion of dehydronorcholene, which was derivable from desoxycholic acid, to methylcholanthrene. The latter was shown[11] to be strongly carcinogenic. However, the validity of the proposal as a biochemical sequence has never been substantiated. One of the difficulties was that at the time the idea was published almost nothing was known about

(1) This investigation was supported in part by Grants E-203 and P-292A of the American Cancer Society and constitutes a portion of the research carried out by D. L. Ford in fulfillment of the requirements for the Ph.D. degree.

(2) A preliminary report of this work has appeared: W. R. Nes and D. L. Ford, *Tetrahedron Letters*, **No. 5**, 209 (1962).

(3) Recipient of National Science Foundation Graduate Fellowship, September, 1961, to June, 1962.

(4) E. Clar, *Ber.*, **62**, 350 (1929).

(5) E. Kennaway and I. Hieger, *Brit. Med. J.*, **1**, 1044 (1930).

(6) E. Kennaway, *Biochem. J.*, **24**, 497 (1930).

(7) J. W. Cook, C. L. Hewett and I. Hieger, *J. Chem. Soc.*, 395 (1933).

(8) O. Rosenheim and H. King, *Nature*, **130**, 315 (1932); *Chem. Ind.* (London), **51**, 954 (1932).

(9) H. Wieland and E. Dane, *Z. physiol. Chem.*, **210**, 268 (1932).

(10) E. Kennaway and J. W. Cook, *Chem. Ind.* (London), 521 (1932).

(11) J. W. Cook and G. A. D. Haslewood, *J. Chem. Soc.*, 428 (1934); *cf. Chem. Ind.* (London), 758 (1933).

(12) H. Wieland and E. Dane, *Z. physiol. Chem.*, **219**, 240 (1933).

the metabolism of steroids, and it was not possible to make a detailed analysis of probable biochemical transformations.

More recently, research on the metabolism of steroids has evolved a wealth of information, and it is our feeling that the essence of the proposal, *viz.*, that a steroid proceeds to a polycyclic aromatic carcinogen, should not be abandoned until it has been placed and tested within the framework of modern biochemistry.

In 1953, the suggestion[13] was made that the anthrasteroid rearrangement might allow a route from a steroid to such a carcinogen, and a modification of the closely similar dienone–phenol rearrangement has, in the meantime, been shown[14,15] to occur biologically. The anthrasteroid rearrangement disposes of the necessity for removing an angular methyl group (C-19) and yields a ring system characteristic of the 10-methyl-1,2-substituted-anthracenes.

The purpose of the present paper is to show that an anthrasteroid can be converted to 4′,10-dimethyl-1,2-benzanthracene by a sequence of steps which parallel known types of biochemical transformations, and this sequence is offered as a model of what might happen biologically.

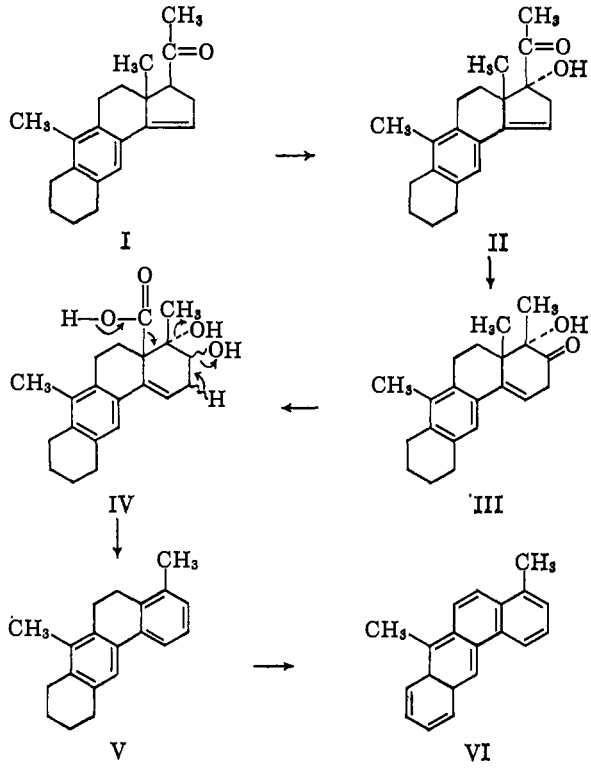
The Model.—The conversion of an anthrasteroid to a benzanthracene requires three major conversions: (a) enlargement of ring-D, (b) removal of the remaining angular methyl group (C-18), and (c) the elimination of several hydrogen atoms.

The first of these conversions should be capable of attainment from a 20-ketoanthrasteroid (I) by 17α-hydroxylation to II and D-homosteroid rearrangement to III. From experiments which began with the isolation of corticosterone[16,17] (and similar hormones) and the demonstration[18] that it arises from pregnenolone in perfused adrenals, it is now well established that 17α-hydroxylation of 20-ketosteroids is a biochemical reaction. Evidence for the biochemical D-homorearrangement has also been brought forward in both microörganisms[19] and mares.[20]

The removal of the angular methyl group could reasonably occur biochemically by oxidation of C-18 to the carboxyl stage (known to occur in mammals from the isolation[21] of the C-18 acid of corticosterone) and reduction of the 17-keto group by a pyridine nucleotide to a carbinol group (III to IV). By a concerted *trans*-β-elimination of CO_2 and the 17aα-hydroxyl group, C-18 would be lost (IV to V). Precisely this kind of elimination is known biochemically in the conversion[22] of mevalonic acid pyrophosphate to Δ^2-isopentenyl pyrophosphate under the influence of adenosine triphosphate which suffers cleavage to ADP and phosphate ion. In the case of the anthra-D-homosteroid, the product, by dehydration to V and introduction of additional double bonds, could then yield 4′,10-dimethyl-1,2-benzanthracene (VI). One reasonable way in which the final double bonds might arise is by hydroxylation–dehydration. This kind of route is known biochemically in the aromatization, for instance, of proline to pyrrole-2-carboxylic acid.[23] Direct dehydrogenation, *e.g.*, by a quinone (ubiquinone, etc.), is also conceivable; although there is not known to us a good biochemical example of direct dehydrogenation of a substrate lacking a carbonyl group, chloranil will aromatize steroids in organic solvents.[24]

SCHEME 1

PROPOSED BIOCHEMICAL REACTIONS



In order to show that an anthrasteroid is intrinsically capable of conversion to a benzanthracene by 17α-hydroxylation, D-homorearrangement and aromatization, we have carried out such a sequence non-biologically. $\Delta^{5,7,9,14}$-Anthrapregnatetraen-20-one (I), the preparation of which from two different steroids (pregnenolone,[25] ergosterol[26]) has already been described, upon reduction yielded $\Delta^{5,7,9}$-anthrapregnatrien-20-one (VII) which, after conversion to the enol acetate VIII, was 17α-hydroxylated with osmium tetroxide to give IX. When IX was submitted to the base-catalyzed D-homorearrangement, the major product was the 17aβ-hydroxy-17aα-methyl-17-ketone (X) which, upon reduction and acetylation, yielded 17ξ,17aβ-diacetoxy-17aα - methyl - $\Delta^{5,7,9}$ - anthra - D - homoandrostatriene (XIII). The latter was smoothly converted to 4′,10-dimethyl-1,2-benzanthracene (VI) by pyrolysis over Pd–C, and the product was identical with a sample of VI prepared by total synthesis from benzanthraquinone by a modification of the route previously reported.[27,28]

4′,10-Dimethyl-1,2-benzanthracene (VI) is currently under study for its carcinogenicity. While 4′-substituted benzanthracenes frequently are not carcinogenic,

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(17) H. L. Mason, W. M. Hoehn, B. F. McKenzie and E. C. Kendall, *J. Biol. Chem.*, **120**, 719 (1937).

(18) O. Hechter, A. Zaffaroni, R. P. Jacobsen, H. Levy, R. W. Jeanloz, V. Schenker and G. Pincus, *Rec. Progr. Hormone Res.*, **6**, 215 (1951).

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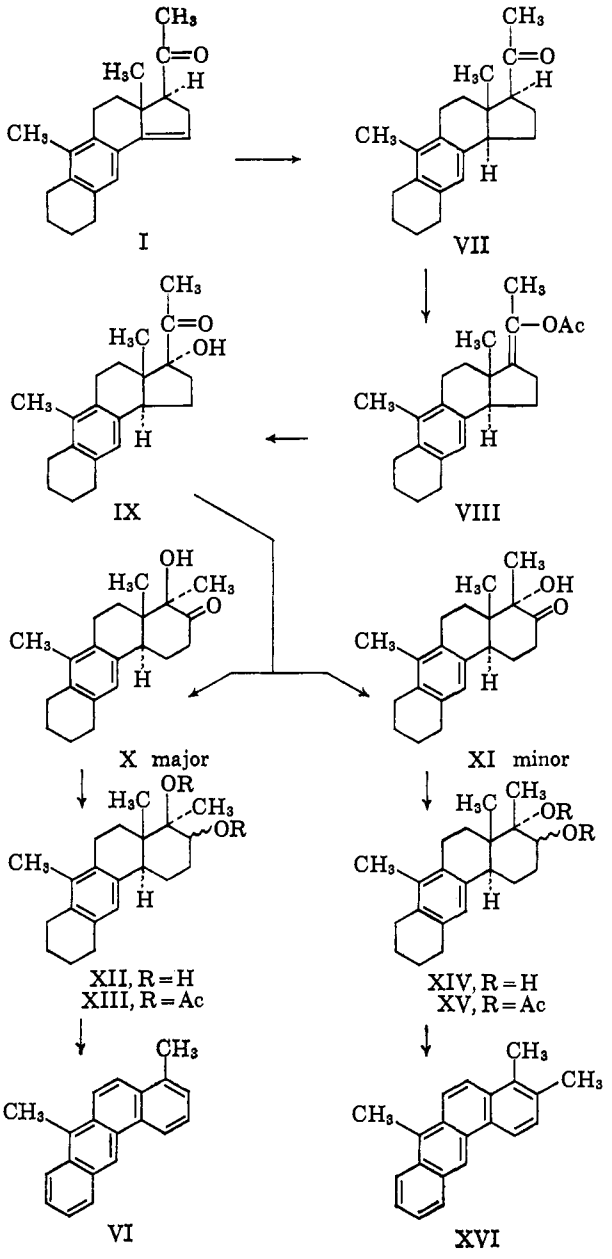
SCHEME 2

REACTIONS ACCOMPLISHED NON-BIOCHEMICALLY



4′,9,10-trimethyl-1,2-benzanthracene, which is very closely related to VI, has been found[29] to induce tumors by skin-painting.

One of several alternative possibilities for a biochemical process is for the ketone III to proceed by reduction to the diol (14-dehydro derivative of XIV) which by rearrangement through the 17a,17a-dimethyl derivative could yield ultimately the 3′,4′,10-trimethyl-1,2-benzanthracene (XVI) for which evidence was obtained in the course of the non-biological work. The latter pathway would not require oxidative elimination of C-18.

Experimental

$\Delta^{5,7,9}$-Anthrapregnatrien-20-one (VII from I).—To a solution of 455 mg. of $\Delta^{5,7,9,14}$-anthrapregnatetraen-20-one[25,26] (I) in 29 ml. of ethyl acetate containing 6 drops of pyridine was added 143 mg. of 10% palladium-on-charcoal, and the mixture was magnetically stirred in a hydrogen atmosphere. After 1 hr. one molecular equivalent of hydrogen was absorbed, but stirring was continued for several hours further in order to remove all traces of I. Removal of the catalyst by filtration with Celite and evaporation of the solvent left a crystalline residue which after two recrystallizations from methanol yielded 369 mg. of colorless needles, m.p. 120–123°. The analytical sample melted at 122–123°, $[\alpha]D$ −87°; λ_{max} 273, 278, and 283 mμ (ε 650, 540 and 675, respectively); λ_{max} 5.88, 6.24 and 6.36 μ.

Anal. Calcd. for $C_{21}H_{28}O$ (296.4): C, 85.08; H, 9.52. Found: C, 84.80; H, 9.56.

The same compound VII was obtained by reduction of the ethylene ketal[26] of I with PtO_2–H_2 in ethyl acetate–acetic acid followed by hydrolysis.[26]

20-Acetoxy-$\Delta^{5,7,9,17(20)}$-anthrapregnatetraene (VIII from VII).—A solution of 365 mg. of VII and 230 mg. of *p*-toluenesulfonic acid in 65 ml. of freshly distilled acetic anhydride was boiled under N_2 for 5.5 hr. with slow distillation. The original volume was maintained by periodic addition of acetic anhydride. The dark brown mixture was cooled to 0°, diluted with 65 ml. of ether, and then extracted with 30 ml. of aq. 5 *N* NaOH. The organic layer was washed with H_2O and evaporated to dryness leaving an oil which was chromatographed on 18 g. of silica gel. Benzene eluted VIII which after crystallization from acetone formed as colorless needles, m.p. 138–143°, 111 mg. Two recrystallizations raised the melting point to 150–151°. The latter sample showed a weak band at 5.90 μ and a strong band at 5.75 μ. The relative areas of the two bands indicated 13% of starting material VII as a contaminant. Separation of the contaminant was not possible by chromatography an alumina, magnesium trisilicate or silica gel, nor by recrystallization from various solvents. Longer times of reaction did not prove worthwhile either.

Anal. Calcd. for $C_{23}H_{30}O_2$ (338.5) (VIII): C, 81.61; H, 8.93. Calcd. for 87% VIII and 13% VII: C, 82.06; H, 9.01. Found: C, 82.20; H, 9.26.

The enol acetate could not be selectively epoxidized in the side chain by perbenzoic acid in benzene. It consumed 4.6 molar equivalents in 20 hr. Under the same conditions $\Delta^{5,7,9}$-anthraergostatriene[13] consumed 3.1 equivalents in 2.5 hr., and it appears the polyalkyl substituted benzenoid ring is more readily attacked than is the $\Delta^{17(20)}$-bond.

Attempts to obtain an enol acetate of the 15-bromo derivative of I failed to give a definitive product.

17α-Hydroxy-$\Delta^{5,7,9}$-anthrapregnatrien-20-one (IX from VIII).—A solution of 250 mg. of osmium tetroxide in 4.0 ml. of dioxane was added to a solution of 232 mg. of the enol acetate VIII in 6.0 ml. of dioxane, and the mixture was allowed to remain at room temperature in the dark for 3 days during which time 750 mg. of additional OsO_4 was added at intervals. The mixture was saturated with H_2S and filtered, and the residue remaining after removal of the solvent was chromatographed on silica gel. Unchanged enol acetate VIII was eluted with benzene and the 17α-hydroxyl product IX was eluted with 10% ether in benzene. Crystallization from petroleum ether yielded 70 mg. of colorless, fluffy needles, m.p. 129–131°. The analytical sample obtained by recrystallization from methylcyclohexane melted at 131–133°, $[\alpha]D$ −63°; λ_{max} 273.0, 277.5 and 282.0 mμ (ε 706, 606 and 766, respectively; $\lambda_{max}^{CS_2}$ 2.88, 5.87 and 5.93 μ; λ_{max}^{KBr} 2.88 and 5.93 μ. The split in the carbonyl band in CS_2 is in agreement with the spectra[30a] of other 17α-hydroxy-20-keto compounds and presumably arises from an equilibrium between molecules with and without the OH group hydrogen bonded to the carbonyl group. The spectrum in KBr indicates complete hydrogen bonding, since only the band at the longer wave length was present.

Anal. Calcd. for $C_{21}H_{28}O_2$ (312.4): C, 80.73; H, 9.03. Found: C, 80.49; H, 9.35.

D-Homorearrangement.—The hydroxy ketone IX (138 mg.) was dissolved in 70 ml. of 5% KOH in methanol, and the mixture was refluxed under N_2 for 10.5 hr. after which the solvent was removed at reduced pressure. The residue was partitioned between water and chloroform, and the residue from the organic layer was a pale yellow oil showing an infrared spectrum which was a composite of the spectra (especially in the 2.8–3.0 and 8.6–9.5 μ regions) of the two epimers separable by partition chromatography. In a ligroin–propylene glycol system[31] of solvents the major component X ($\lambda_{max}^{CS_2}$ 2.89, 5.88, 8.65, 8.98 and 9.41 μ) moved somewhat more than twice as fast as the minor component XI ($\lambda_{max}^{CS_2}$ 2.80, 2.89, 5.85, 8.86, 9.02 and 9.40 μ). Upon adsorption chromatography of the mixture, the fast-moving edge of the elution band possessed an infrared spectrum the same as that of XI and the slow-moving edge a spectrum nearly the same as that of X.

The epimeric mixture (138 mg.) was dissolved in 4.0 ml. of ether and added to 6.0 ml. of 0.6 *M* ethereal $LiAlH_4$. After being refluxed for 5 min. the excess reagent was destroyed with moist ether, and the precipitate was dissolved with 20% aq. NaOH. The ether-soluble material ($\lambda_{max}^{CS_2}$ 2.79, 2.91, 9.07,

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TABLE I

ULTRAVIOLET ABSORPTION[a] OF 1,2-BENZANTHRACENE DERIVATIVES

Derivative	Band in mμ above and ε below										
	A	B	C	D	E	F	G	H	I	J	K
4',10-DiMe (VI)[b]	259.0	275.5	286.0	297.5	...	323.0	338.0	354.4	372.5	...	392.0
by total syn.	34,200	38,250	77,500	93,400	...	5000	8000	10,100	7500	...	545
4',10-DiMe (VI)[c]	259.0	275.5	286.0	297.5	...	323.0	338.0	354.5	372.5	...	392
from I	36,700	41,500	84,000	101,000	...	5300	8500	10,900	8100	...	600
4'-Me[d]	255.0[e]	272.0	282.0	293.0	...	...	328.0	344.0	362.0[e,f]	...	387.0
	[g]	[g]	[g]	[g]	...	...	[g]	[g]	[g]	..	[g]
4'-Me[h]	254	272	282	293	...	...	329	344	362[f]	...	387
3',4',10-TriMe	259.0	278.0	288.0	300.0	...	323.0	338.0	355.0	373.0	...	...
(XVI)[i]	28,700	37,500	73,000	81,000	...	5250	7800	9700	7050	...	...
4'-Bromo[j]	254.5	274.0	284.0	295.5	...	314.0	327.0	343.0	359.5	378.0	389.0
	38,800	40,600	80,000	95,200	...	5940	7960	8850	6170	450	750
4',10-Dibromo[k]	259.0	278.0	289.0	300.5	307.0	325.0	340.5	357.5	371.0	...	395.0
	31,600	37,600	75,400	95,800	18,800	5240	8060	10,000	7250	...	855

[a] The spectra were determined in isooctane on a Cary model 14 recording spectrophotometer. [b] Additional bands at 224.5 mμ (ε 37,100) and 234.5 mμ (ε 33,500). [c] Additional bands at 224.5 mμ (ε 39,800) and 234.5 mμ (ε 36,200). [d] Chromatographically least polar derivative from dehydrogenation of XIII. [e] Shoulder. [f] Additional shoulders at 357 and 367 mμ. [g] ε was not obtainable since there was insufficient material to weigh. [h] Reported by R. N. Jones and C. Sandorfy, "The Ultraviolet Absorption Spectra of Methyl-1,2-benzanthracenes, Supplementary Curves and Numerical Data," National Research Council Bulletin No. 4, Ottawa, Can., 1956. [i] Additional bands at 226.0 mμ (ε 37,000) and 235.0 mμ (ε 35,500). [j] Additional band at 231.5 mμ (ε 49,600). [k] Additional bands at 231.5 mμ (ε 34,700), 238.0 (44,500), 268.5 (20,200) and 376 (7150).

9.34 and 9.68 μ) was acetylated (room temp., 16 hr.) in 19 ml. of acetic anhydride and 12 ml. of glacial acetic acid containing 25 mg. of *p*-toluenesulfonic acid. The product was isolated by dilution with water and extraction with ether. Evaporation of the washed and dried organic layer left a residue from which 77 mg. of **17aβ,17ξ-diacetoxy-17aα-methyl-$\Delta^{5,7,9}$-anthra-D-homoandrostatrien-17-one (XIII)** was obtained by crystallization from acetone. The product formed in colorless hexagonal plates which sublimed too rapidly to allow m.p. determination on the Kofler block. In a sealed capillary the analytical sample obtained by two recrystallizations melted at 256–257°, $[\alpha]_D$ +75°; λ_{max} 273.0, 277.0 and 282.0 mμ (ε 640, 525 and 640, respectively); λ_{max}^{KBr} 5.77, 7.91, 8.00 and 8.13 μ.

Anal. Calcd. for $C_{25}H_{34}O_4$ (398.5): C, 75.34; H, 8.60. Found: C, 75.43; H, 8.70.

That D-homorearrangement of IX yielded 17-ketones expected from the work of Turner[32,33] and Wendler[34–36] and not the isomeric and less stable[36] 17a-ketones was established by a positive Zimmermann test. The 17-ketones possess the necessary CH_2– group adjacent to a carbonyl group, but the 17a-carbonyl group would be flanked by two fully substituted carbon atoms (C-13 and C-17). Mechanistic considerations[32,33] also predict that the major product will be the epimer with the 17a-hydroxyl group β-oriented. In agreement with this, we found that the major product possessed a single absorption maximum at 2.89 μ, while the minor product exhibited two bands at 2.80 and 2.89 μ as has been found[34] for other D-homoketols with equatorially and axially oriented hydroxyl groups, respectively. The carbonyl group in the major product also absorbed at a lower frequency than did that of the minor product, as expected.[34,37]

Dehydrogenation.—The diacetate XIII (47 mg.) was brought into intimate contact with 47 mg. of 5% palladium-on-charcoal by briefly shaking a mixture of the two materials with ether followed by evaporation of the solvent. Dehydrogenation was then carried out in a glass tube which was continuously flushed with CO_2. The gases were collected over 50% aq. KOH. Over a period of 2.5 hr. the temperature was raised from an initial 207 to 322° and 10.5 ml. of gas was collected at 748 mm. pressure and 27°. This volume (which is corrected for the presence of 0.5 ml./hr. of collectable gas from the CO_2) represents 80% of 4 molar equivalents. The reaction tube was cooled, and the sublimate in the upper portion was brought again into contact with the catalyst by washing with ether. After removal of the solvent, heating was continued at 328–348° for 1 hr. during which time the additional gas collected raised the total to 97% of 4 molar equivalents.

The products (16 mg.) were separated from the catalyst by extraction with ether; extraction with several other solvents yielded an additional 4 mg. The combined extracts were chromatographed on silica gel (Davison 923) deactivated[38] with 3% of H_2O, and then appropriate fractions were rechromatographed on alumina (Woelm, neutral, activity grade I) which was deactivated with 3% of H_2O. Each fraction was analyzed by ultraviolet spectroscopy, and combination of appropriate ones from the second chromatogram yielded 11.2 mg. (37%) of **4',10-dimethyl-1,2-benzanthracene (VI)** which melted at 151–153° after crystallization from ethanol. Two recrystallizations from ethanol yielded thick needles, m.p. 153.5–155.0°, with changes in crystalline structure prior to melting. An additional slow recrystallization yielded a mixture of thick needles and at least one other kind of crystal, but the melting point (154–156°) and prior melting behavior were not greatly altered. The changes in crystalline structure observable under the microscope were not readily definable, but they involved growth of new small crystals at 131–142° and some sublimation at 142–154°. The product possessed ultraviolet bands as shown in Table I. In addition, it exhibited ν_{max} at 3075(weak), 2940(weak), 1622(weak), 1545(weak), 877(strong), 805(strong), and 732(strong) cm.$^{-1}$. Infrared bands of this sort are in agreement with earlier generalizations[30b] of the spectra of benzanthracenes.

Anal. Calcd. for $C_{20}H_{15}$ (256.3): C, 93.71; H, 6.29. Found: C, 93.52; H, 6.40.

The over-all yield of crystalline VI (m.p. 151–153°) from the 17α-hydroxy compound IX was 17% (or 23% when calculated on the basis of the total amount of VI in the final chromatographic fractions). The recrystallized material (m.p. 154–156°) did not depress the melting point of the material prepared by total synthesis.

The chromatography of mixtures from dehydrogenation was carried out in several different ways. For analytical purposes the silica gel column was eluted with cyclohexane and ether which allowed direct spectroscopic analysis. Thus, with 2.5 mg. of dehydrogenation product on 6.0 g. of 3% deactivated silica gel there was a progression of elution of naphthalenes (strongest λ_{max} 235 mμ), then anthracenes (strongest λ_{max} 262 mμ), then benzanthracenes (strongest λ_{max} 298 mμ) as the eluent was changed slowly through 250 ml. from pure cyclohexane to 1% ether. From 1% to 5% ether (100 ml.) naphthalenes and anthracenes were again removed but together; the latter presumably still contained acetoxyl groups. *n*-Hexane and ether was an even better system of solvents for separation of the components, but an impurity in our *n*-hexane prevented spectroscopic measurements below 270 mμ, and each fraction had to be evaporated to dryness and redissolved in cyclohexane. It was, however, *n*-hexane which we used for preparative scale (*ca.* 20 mg.) work. A compound showing the spectrum (Table I) of **4'-methyl-1,2-benzanthracene** was eluted just prior to the elution of 4',10-dimethyl-1,2-benzanthracene, but the yield (spectroscopic) of the former was less than 1% and did not allow identification as a solid. However, from 20 mg. of dehydrogenation product, the silica gel chromatography afforded 14 mg. of crystalline benzanthracenes. Rechromatography of the latter on neutral alumina (15 g.), deactivated with 3% of water, by elution with 400 ml. of solvent graded from hexane to 5% ether in hexane yielded a barely detectable amount of the monomethylbenzanthracene (λ_{max} 294 mμ) at the leading edge of the main elution band which possessed λ_{max} 298 mμ. Following the main

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(36) N. L. Wendler, D. Taub and R. W. Walker, *Chem. Ind.* (London), 903 (1959).

(37) D. K. Fukushima, S. Dobriner, M. S. Heffler, T. H. Kritchevsky, F. Herling and G. Roberts, *J. Am. Chem. Soc.*, **77**, 6585 (1955).

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band there was a band exhibiting λ_{max} 300 mμ. Combination of the fractions of the main band yielded the sample of VI discussed above.

From a similar dehydrogenation of the mother liquors of XIII and similar chromatography there was obtained, from the elution band with λ_{max} 300 mμ, 5 mg. of substance believed to be **3′,4′,10-trimethyl-1,2-benzanthracene (XVI)**. Crystallization from ethanol gave colorless plates, m.p. 164–166°. Several recrystallizations raised the melting point to 167–169°; $\nu_{max}^{CCl_4}$ 3070, 2930, 2865, 876 and 683 cm.$^{-1}$; $\nu_{max}^{CS_2}$ 816, 738 and 803 cm.$^{-1}$. For ultraviolet data see Table I. Insufficient material was available for combustion analysis which we felt was less valuable than the spectroscopic characterization for which our sample was used.

Total Synthesis of 4′,10-Dimethyl-1,2-benzanthracene (VI).—The sequence used was that previously reported[28] from 4′-bromobenzanthraquinone,[27] except that it was found preferable to modify the reduction[27,28] of the latter in the following way.

A mixture of 20 g. of 4′-bromobenzanthraquinone in 2 l. of glacial acetic acid and 200 ml. of concd. hydrochloric acid containing 40 g. of stannous chloride was refluxed 75 min. After cooling and addition of 3 l. of ice, the precipitated bromobenzanthrone was dried under a vacuum over P_2O_5 for 3 days. One-half of the product (19.3 g.) was dissolved in 500 ml. of tetrahydrofuran, and then 1 l. of glacial acetic acid and 100 g. of zinc dust was added. The mixture was refluxed with stirring for 2.5 hr. (which, from rough kinetic studies, proved to be an optimal time), cooled and filtered. The filtrate was then diluted with a large volume of water and extracted several times with ether. The ether extract was washed with H_2O and then with 10% aq. NaOH. The residue from the ether extracts was chromatographed on 750 g. of neutral alumina in *warm* benzene. Seventy fractions of 20 ml. were collected. The residue from fractions 31–70 upon crystallization from glacial acetic acid yielded colorless **4′-bromo-1,2-benzanthracene** as hexagonal plates, m.p. 213–215°, lit.[27] m.p. 210–211°. The yield of crystallized material was 10.2 g. after both halves of the crude bromobenzanthrone were carried through the zinc reduction and chromatography. Several alternatives, *e.g.*, reduction with metal hydrides, to the zinc reduction did not prove satisfactory.

The 4′-bromo-1,2-benzanthracene by bromination[28] at position 10 and replacement[28] of the halogens with lithium from butyllithium and then with methyl iodide yielded 4′,10-dimethyl-1,2-benzanthracene (VI), m.p. 154.5–156.0°, lit.[28] m.p. 154–154.5°. The ultraviolet spectrum of VI prepared in this way is given in Table I, and the polymorphism and behavior on heating were identical to the sample prepared from the anthrasteroid. Table I also records the spectra of the brominated intermediates.

Spectroscopic Correlations.—From known spectra of methyl- and dimethyl-1,2-benzanthracenes, the bathochromic shift associated with new substitution can be anticipated for the so-called D- and H-bands.[39,40] The change from no substitution (1,2-benzanthracene, D = 287.5 mμ, H = 341.0 mμ) to 4′-methyl substitution is +5.5 mμ for the D-band and +3.5 mμ for the H-band. For 10-methyl substitution it is +4.0 and +13.5 mμ, respectively. For disubstitution, the values for the D-band are known to be additive plus 0.5–1.5 mμ for the 5,10-, 8,10- and 9,10-dimethyl derivatives. For the H-band they are additive minus 1–3 mμ. Thus, the calculated values for the D-band of **4′,10-dimethyl-1,2-benzanthracene** are 287.5 + 5.5 + 4.0 + (0.5 to 1.5) = 297.5–298.5 mμ. The observed value is 298 mμ. For the H-band calculation gives 341.0 + 3.5 + 13.5 − (1 to 3) = 355 to 357 mμ. The observed value is 354.5 mμ.

The dehydrogenation of the crystalline diacetate XIII also gave in yields of *ca.* 0.7% two benzanthracenes, one of which chromatographed ahead of and one behind VI. The faster moving had an ultraviolet spectrum identical with **4′-methyl-1,2-benzanthracene** (Table I), and the slower possessed a spectrum indicating, on the basis of the D-band at 300 mμ and the H-band at 355 mμ, that it was at least 4′,10-disubstituted. Since it was different from VI, we assign to it the structure of **3′,4′,10-trimethyl-1,2-benzanthracene (XVI)** in keeping with the observation that the yield was increased tenfold (to 5–9%) when mother liquor material (in which the 17aα-acetoxy compound XV was enriched) from the crystalline diacetate was dehydrogenated. 1,2-Diaxial orientation of the angular methyl and 17a-acetoxyl groups of XV would be expected to favor rearrangement. From the known shift for 3′-substitution[41] for the D-band (+1.5 mμ) and H-band (+2.5), the calculated positions (uncorrected for multiple substitution) for XVI are 298 + 1.5 = 299.5 and 354.5 + 2.5 = 357 mμ, respectively, in excellent agreement with observation when it is remembered (foregoing paragraph) that for multiple substitution the D-band is generally at a wave length slightly greater than calculated and the H-band slightly less than calculated. Trisubstitution was verified from the infrared spectrum (in CCl_4) of XVI which exhibited an increased ratio of alkyl C–H stretching to aromatic C–H stretching absorption. For the dimethyl (VI) and trimethyl (XVI) derivatives, the ratio of alkyl to aromatic H-atoms is 6/10 = 0.60 and 9/9 = 1.0, respectively, and the first ratio divided by the second is 0.60. Experimentally, the ratio of the integrated intensities of the respective bands (near 2935 and 2870 cm.$^{-1}$ and near 3071 cm.$^{-1}$) for VI was 2.3 and for XVI was 3.6, and 2.3/3.6 = 0.64 in excellent agreement with theory.

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(41) For the data for this and other substituted 1,2-benzanthracenes see ref. 39, 40 and *h* in Table I.

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Biosynthesis of the Nicotiana Alkaloids. IX. The Non-random Incorporation of Acetate-2-C^{14} into the Pyridine Ring of Anabasine[1]

BY ALAN R. FRIEDMAN[2] AND EDWARD LEETE[3]

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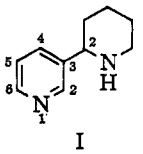
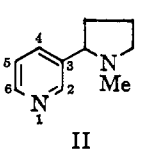
Radioactive anabasine (2-(3-pyridyl)-piperidine) produced when sodium acetate-2-C^{14} was injected into the stems of intact *Nicotiana glauca* plants had 37% of its activity located in the pyridine ring. Using a new degradative scheme it was established that all this activity was located at C_2 and C_3 and was divided approximately equally between these positions. The significance of this result and its relation to studies on the biosynthesis of nicotine and ricinine is discussed.

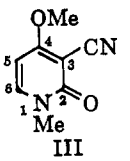
It has been established that the pyridine ring of anabasine (I)[4] and nicotine (II)[5] and the pyridone ring of ricinine (III)[6,7] are derived from nicotinic acid. However, until recently, the biosynthesis of nicotinic acid in the plants which produce these alkaloids has remained a mystery. It was established[8–10] some time



I



II



III

ago, that tryptophan, the precursor of nicotinic acid in animals and some microörganisms, is apparently not converted to nicotinic acid in higher plants. Five years ago we[11] and Byerrum and Griffith[12] showed that

(1) This investigation was supported by a research grant (MY-02662) from the National Institutes of Health, U. S. Public Health Service. Part VIII in this series: E. Leete and V. M. Bell, *J. Am. Chem. Soc.*, **81**, 4358 (1959).

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