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Mechanisms of Steroid Oxidation by Microörganisms.[1] II. Isolation and Characterization of 3aα-H-4α-[3′-Propionic acid]-7aβ-methylhexahydro-1,5-indanedione

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Androst-4-ene-3,17-dione was converted to 3aα-H-4α-[3′-propionic acid]-7aβ-methylhexahydro-1,5-indanedione (II) by exposure to *Nocardia restrictus*. The structure of the acid II was established by asymmetric synthesis. (±)-*anti-trans*-1β-Hydroxy-8β-methyl-4,5-(4-keto-1,2,3,4-tetrahydrobenzo)-hydrindane (VI) was oxidized to (+)-*anti-trans*-1-keto-8β-methyl-4,5-(4-keto-1,2,3,4-tetrahydrobenzo)-hydrindane (VII) with *Pseudomonas testosteroni*; ozonolysis of VII gave the acid II.

One pathway of steroid degradation by microörganisms involves a 9α-hydroxylation followed by a 1,2-dehydrogenation or *vice versa* with the rupture of the steroid ring B.[2] The initial degradative reactions may be visualized as: androst-4-ene-3,17-dione → 9α-hydroxyandrost-4-ene-3,17-dione or androsta-1,4-diene-3,17-dione → 3-hydroxy-9,10-*seco*-androsta-1,3,5-(10)-triene-9,17-dione (I) ⟶ $CO_2 + H_2O$. Progesterone is degraded similarly *via* the corresponding 9,10-*seco*-phenol to 7aβ-methyl-1-acetylhydrindan-5-one-[β-propionic acid-(4)] by *Mycobacterium smegmatis*.[3] This paper reports the isolation, characterization and synthesis of 3aα-H-4α-[3′-propionic acid]-7aβ-methylhexahydro-1,5-indanedione (II). This acid is formed when I is incubated with *Nocardia restrictus*.

Incubation of androst-4-ene-3,17-dione with *N. restrictus* produced besides the 9,10-*seco*-phenol (I) a new acid II, m.p. 110–111.5°. This compound was initially recognized as an acid by its solubility in sodium bicarbonate and its reaction with brom thymol blue indicator. The infrared spectrum in Nujol of its potassium salt showed the presence of a five-membered ring carbonyl group (5.75 μ), a six-membered ring carbonyl group (5.86 μ) and a carboxylate ion (6.32 μ). Non-aqueous titrations gave a neutralization equivalent of 236 and 239 in two separate determinations, and the carbon hydrogen analysis was consistent with $C_{13}H_{18}O_4$. The n.m.r. spectrum of the compound showed a band at 8.79 τ (3H, one tertiary CH_3). These results indicate that ring A of the steroid probably has been degraded into a hydrindane propionic acid.

Because 7aβ-methyl-1-acetylhydrindan-5-one-[β-propionic acid-(4)] was characterized by refluxing with acetic anhydride to form an enol lactone,[3] a similar approach to the elucidation of the structure of II was taken. Unfortunately when II was refluxed with acetic anhydride, a mixture of three products was formed. One of these (R_f 0.60) was assigned the structure VIII on the basis of the following results: Carbon and hydrogen analysis were in good agreement with $C_{15}H_{20}O_5$; the infrared spectrum showed the presence of a five-membered ring carbonyl (5.75 μ) and a six-membered ring lactone with a negative α-substituent (5.65 μ)[4]; the n.m.r. spectrum showed bands at 8.97 τ (3H, one tertiary CH_3) and 7.92 τ (3H, CH_3COO^-) with no bands in the region characteristic of vinylic protons; the absence of a free carboxyl group was shown by its reaction with brom thymol blue indicator and its mobility on paper chromatograms (carboxylic acids have much lower mobilities); compound VIII could easily be hydrolyzed with methanolic potassium hydroxide at room temperature back to II. The other principal product (R_f 0.76) was obtained as an oil whose infrared spectrum showed a broad band around 5.80 μ. No attempt was made to isolate the third component (R_f 0.26) as the yield was extremely low. When II was heated with acetic anhydride at 60° or refluxed in the presence of an acidic catalyst such as acetyl chloride, similar mixtures of these products were obtained.

Treatment of II with acetic anhydride and sodium acetate[5] afforded 3aα-H-4α-[3′-propionic acid]-5-hydroxy-7aβ-methyl-3a-4,7,7a-tetrahydro-1-indanone-δ-lactone (III). The position of the double bond in this compound ($\Delta^{5,6}$ rather than $\Delta^{4,5}$) was assigned initially on the basis of its infrared spectrum, which showed the presence of a band at 6.02 μ.[6] This assignment was confirmed by the presence of a band corresponding to an isolated proton on a double bond in the n.m.r. spectrum of the compound, 4.72 τ (1H, one vinylic proton) and 9.05 τ (3H, one tertiary CH_3). On the basis of the relative stabilities of Δ^2-cholestene and Δ^3-cholestene[7] and the direction of enolization of the 9-keto function in 9,10-*seco*-phenol I,[2] the double bond in III would be expected to occupy the $\Delta^{5,6}$-rather than the $\Delta^{4,5}$-position.

Reduction of II with sodium borohydride resulted in the formation of a dihydroxy acid (IV). The 5β-configuration was assigned to the hydroxyl of this acid, for metal hydride reduction of unhindered ketones yields the thermodynamically stable, equatorial isomer.[8] Attempts to lactonize the dihydroxy acid IV by the use of *p*-toluenesulfonic acid in either benzene, toluene or xylene[9] were unsuccessful. However, a lactone acetate (V) was readily obtained when IV was heated with acetic anhydride and sodium acetate. Since these evidences are only indicative, we undertook a more definitive proof of the structure by asymmetric synthesis. Incubation of (±) *anti-trans*-1β-hydroxy-8β-methyl-4,5-(4-keto-1,2,3,4-tetrahydrobenzo)-hydrindane (VI)[10] with *Pseudomonas testosteroni*[11] resulted in the formation of (+)-*anti-trans*-1-keto-8β-methyl-4,5-(4-keto-1,-2,3,4-tetrahydrobenzo)-hydrindane (VII) in 90% yield. Ozonolysis of VII afforded the acid II, identical in all respects (m.p., m.m.p., infrared spectrum) with a sample of II obtained from *Nocardia restrictus* fermentation. This sequence of reactions conclusively established the structure of II as 3aα-H-4α-[3′-propionic acid]-7aβ-methylhexahydro-1,5-indanedione.

(1) This investigation was supported by research grants (AM-04874-02) and (A-6110) from the U. S. Public Health Service.

(2) R. M. Dodson and R. D. Muir, *J. Am. Chem. Soc.*, **83**, 4627 (1961).

(3) K. Schubert, R. H. Bohme and C. Horhold, *Z. physiol. Chem.*, **325**, 260 (1961).

(4) The γ-acetoxy-γ-valerolactone also exhibits its lactone C=O band at a shorter wave length (5.56 μ) than normal. This has been explained by assuming an inductive effect on the lactone C=O band position because of negative α-substituents (acetoxy group) on the alcohol part of the molecule (R. S. Rasmussen and R. R. Brattain, *J. Am. Chem. Soc.*, **71**, 1073 (1949).

(5) G. I. Fujimoto and J. Prager, *ibid.*, **75**, 3259 (1953).

(6) The position of the double bond in the enol lactone reported by Schubert, *et al.*, in ref. 3 was not assigned and the infrared spectrum showed no bands in the region of 5.9–6.1 μ.

(7) R. B. Turner, W. R. Meador and R. E. Winkler, *J. Am. Chem. Soc.*, **79**, 4122 (1957).

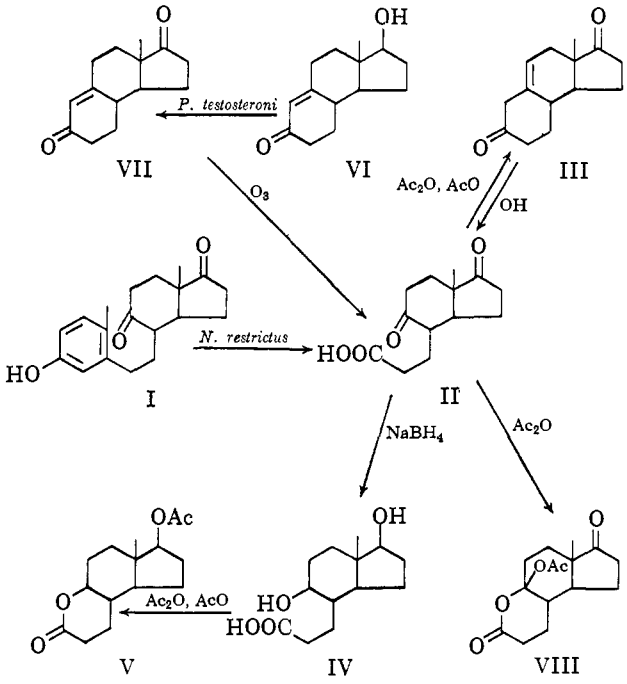
(8) D. H. R. Barton, *J. Chem. Soc.*, 1027 (1953).

(9) W. S. Johnson, V. J. Bauer, J. L. Margrave, M. A. Frisch, L. H. Dreger and W. N. Hubbard, *J. Am. Chem. Soc.*, **83**, 606 (1961).

(10) J. Chinn and H. L. Dryden, *J. Org. Chem.*, **26**, 3904 (1961).

(11) This organism was selected for the dehydrogenation of VI as this organism in small scale experiments gave better yields of VII than all the other organisms tested.

When the 9,10-*seco*-phenol I was exposed to *N. restrictus* a rapid and high yield conversion to the acid II was observed. Similar transformation of I into II was noted with *Bacterium cyclo-oxydans*, *Pseudomonas testosteroni* and *Mycobacterium rhodochrous*, indicating that this is a general process of steroid degradation among microörganisms. The following compounds under similar conditions were not attacked: estrone, 1-methylestrone, 1-hydroxy-4-methyl-3-deoxyestrone and 3-hydroxy-9,10-*seco*-estra-1,3,5(10),8(14)-tetraene-9,17-dione. These results show that both the 9,10-*seco* structure and the C-10 methyl group were required for further oxidation of the aromatic ring.



Experimental[12]

Fermentation of Androst-4-en-3,17-dione.—*Nocardia restrictus*[13] No. 545 was grown in 4.8 l. of Difco nutrient broth (12 2-l. erlenmeyer flasks) at 25° on a rotary shaker. After 24 hr. of incubation, 14.4 g. of androst-4-ene-3,17-dione dissolved in 110 ml. of dimethylforamide was distributed equally among the 12 flasks. *It is imperative to use relatively high steroid concentrations (3 mg./ml.); otherwise no products will accumulate.* After 14 hr., the culture broth was acidified with 6 *N* hydrochloric acid (250 ml.) and extracted three times with three 2-l. portions of chloroform. An aliquot of the chloroform extract was spotted on Whatman No. 1 paper and developed in a toluene–propylene glycol system[14] for 3 hr. After drying, the paper was sprayed with the Zimmermann reagent.[15] Two major magenta colored spots appeared with R_f values of 0.28 and 0.07. The combined chloroform extract was dried over sodium sulfate and taken down to dryness to give 6.84 g. of residue. The residue was taken up in 400 ml. of benzene–ether (1:1) and extracted three times with three 75-ml. portions of 6% $NaHCO_3$; the benzene–ether layer was further extracted three times with three 75-ml. portions of 5% NaOH. The bicarbonate fraction was acidified with hydrochloric acid and extracted three times with three 100-ml. portions of chloroform; the chloroform layer was washed with water, dried over sodium sulfate and concentrated to dryness to give 2.42 g. of solids. Since attempts at crystallization failed, the 2.42 g. of residue was chromatographed on 80 g. of silicic acid.[16] The column was washed with chloroform; elution of the column with 1.5% methanol in chloroform afforded 1.81 g. of material which crystallized after trituration with ether. Two recrystallizations from acetone–petroleum ether yielded 1.48 g. of an acid (*II*), m.p. 110–111.5°, $[\alpha]^{25}D$ +121°; $\lambda_{max}^{CHCl_3}$ 3.85, 5.75 and 5.85 μ; the infrared spectrum in Nujol of the potassium salt showed bands at 5.75, 5.86 and 6.32 μ; neutralization equivalent 236 and 239.

Anal. Calcd. for $C_{13}H_{18}O_4$: C, 65.53; H, 7.61. Found: C, 65.78; H, 7.88.

The phenolic fraction was acidified with acetic acid and extracted with 300 ml. of chloroform in three portions. The chloroform extract was washed with water, dried over sodium sulfate and taken down to dryness to give 0.41 g. of residue. Two recrystallizations from acetone–petroleum ether afforded 210 mg. of 3-hydroxy-9,10-*seco*-androsta-1,3,5(10)-triene-9,17-dione (I), m.p. 122–124°, identical in all respects (m.m.p., infrared and ultraviolet spectra and paper chromatography) to an authentic sample.

Reaction of 3aα-H-4α-[3′-Propionic acid]-7aβ-methylhexahydro-1,5-indanedione (II) with Acetic Anhydride.—To 400 mg. of the acid II was added 20 ml. of acetic anhydride and the mixture was refluxed for 1.5 hr. After removing the acetic anhydride the residue was taken up in toluene and chromatographed over a cellulose-powder column[17] using propylene glycol as the stationary phase. Paper chromatography of an aliquot of the toluene extract showed three Zimmermann positive spots with R_f values of 0.76, 0.60, and 0.26. Elution of the column with 20% toluene in cyclohexane afforded 120 mg. of an oily residue whose infrared spectrum showed a broad band at 5.80 μ. Further elution of the column with 70% toluene in cyclohexane yielded 80 mg. of a compound (VIII), m.p. 209–211°, $[\alpha]^{24}D$ +83°, $\lambda_{max}^{CHCl_3}$ 5.65 and 5.75 μ.

Anal. Calcd. for $C_{15}H_{20}O_5$: C, 64.27; H, 7.19. Found: C, 64.48; H, 7.58.

To eliminate the possibility of some unexpected cyclization or rearrangements, a small portion of the above lactol acetate VIII was hydrolyzed, using methanolic potassium hydroxide, back to the starting acid II (identity established by m.m.p. and comparison of infrared spectra).

No attempt was made to isolate the third component (R_f 0.26) as the yield of this product was very low.

3aα-H-4α-[3′-Propionic acid]-5-hydroxy-7aβ-methyl-3a-4,7-7a-tetrahydro-1-indanone-α-lactone (III).—A mixture containing 0.9 g. of the acid II, 0.81 g. of anhydrous sodium acetate and 40 ml. of acetic anhydride was heated in an oil-bath (80–95°) for 48 hr. under an atmosphere of nitrogen. After evaporation of the acetic anhydride the residue was dissolved in 150 ml. of ether. The ethereal solution was washed with two 60-ml. portions of 6% sodium bicarbonate solution and 40 ml. of distilled water in two portions, dried over sodium sulfate and concentrated down to dryness. Three recrystallizations from acetone–petroleum ether afforded 286 mg. of the enol lactone III, m.p. 137–138.5°, $[\alpha]^{25}D$ +286°; $\lambda_{max}^{CHCl_3}$ 5.70, 5.75 and 6.02 μ.

Anal. Calcd. for $C_{13}H_{16}O_3$: C, 70.89; H, 7.32. Found: C, 71.40; H, 7.61.

In order to eliminate the possibility that some unexpected rearrangement had occurred during the enol lactonization, a small portion of the above enol-lactone III was hydrolyzed with ethanolic sodium hydroxide at room temperature back to the starting acid II (identity established by m.m.p. and infrared spectra).

3aα-H-4α-[3′-Propionic acid]-7aβ-methylhexahydro-1β,5β-indanediol (IV).—To 500 mg. of the acid II in 5 ml. of methanol was added 240 mg. of sodium borohydride dissolved in methanol (5 ml.). After the mixture was left standing at room temperature for 16 hr., 1 ml. of glacial acetic acid was added to destroy the excess borohydride. The methanol was then removed and 100 ml. of water was then added; after acidification the reaction mixture was extracted with 150 ml. of chloroform in three portions. The chloroform extract was dried over sodium sulfate and taken down to dryness. Two recrystallizations from acetone–petroleum ether yielded 337 mg. of the dihydroxy acid IV, m.p. 154–155.5°, $\lambda_{max}^{CHCl_3}$ 2.90 and 5.78 μ.

Anal. Calcd. for $C_{13}H_{22}O_4$: C, 64.44; H, 9.15. Found: C, 64.13; H, 8.90.

Lactonization of the Dihydroxy Acid IV.—A mixture containing 0.297 g. of the dihydroxy acid IV, 0.27 g. of anhydrous sodium acetate and 13.3 ml. of acetic anhydride was heated for 48 hr. in an oil-bath (80–95°) under an atmosphere of nitrogen. After

(12) Melting points, determined on a Thomas–Hoover melting point apparatus, are corrected. The rotations were taken in chloroform and have been approximated to the nearest degree. Ultraviolet absorption spectra were determined on a model 11 MS Cary recording spectrophotometer and 95% ethanol was used as solvent. Infrared spectra were recorded on a Beckman IR 5A double beam infrared recording spectrophotometer. Microanalyses and non-aqueous titrations were carried out by Mr. J. Alicino of Metuchen, N. J. "Petroleum ether" refers to the fraction of b.p. 60–80°. All n.m.r. spectra were determined on a Varian Associates recording spectrometer (A60) at 60 Mc. in deuterated chloroform. Chemical shifts are reported in τ-values (p.p.m.) [G. V. D. Tiers, *J. Phys. Chem.*, **62**, 1151 (1958)]. We thank Mr. Roy Matsuo for these determinations.

(13) This organism was kindly supplied by Professor R. Gordon of The Institute of Microbiology, Rutgers University, New Brunswick, N. J.

(14) A. Zaffaroni, R. B. Burton and E. H. Keutman, *Science*, **111**, 6 (1950).

(15) W. Zimmermann, *Z. physiol. Chem.*, **233**, 257 (1935).

(16) The silicic acid (Mallinckrodt 2847) was washed with acetone–ether (2:1) and dried at 90–100°.

(17) C. J. Sih and R. E. Bennett, *Biochim. Biophys. Acta*, **38**, 378 (1960).

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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(18) W. J. Haines and N. A. Drake, *Federation Proc.*, **9**, 180 (1950).

(19) C. J. Sih, *Biochim. Biophys. Acta*, **62**, 541 (1962).

(20) F. Brown, *Biochem. J.*, **47**, 598 (1950).

(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]

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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).