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The Anthrasteroid Rearrangement. V. The Preparation of an Analog of Progesterone[1]

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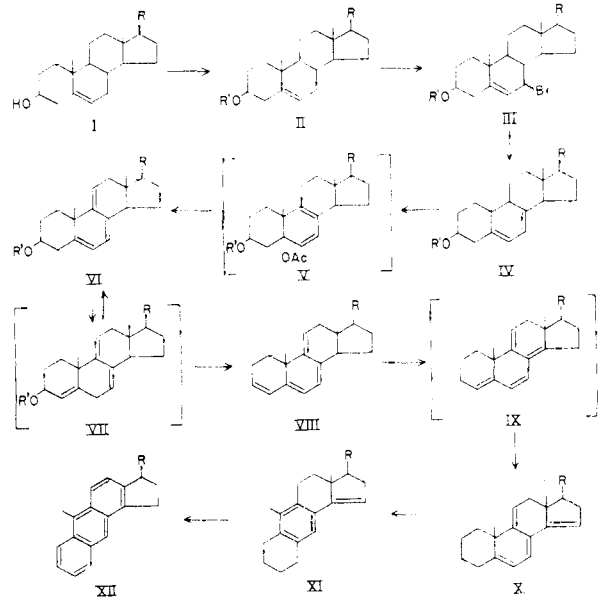
The anthrasteroid, 5,7,9,14-anthrapregnatetraen-20-one (XI, R = $COCH_3$), which bears the side chain of progesterone, has been prepared from pregnenolone for carcinogenic and endocrinologic studies. The structure and nomenclature of anthrasteroids as a class are discussed.

It was suggested in earlier publications[2] from this Laboratory that the anthrasteroid rearrangement may form part of a biochemical pathway to endogenous carcinogens, and since then this hypothesis has received the attention of several other authors.[3] Various anthrasteroids, *viz.*, XI (R = C_9H_{17} and C_8H_{17} derived, respectively, from ergosterol and cholesterol), the 14,15-dihydro derivatives of XI (R = C_9H_{19} and C_8H_{17}), and the corresponding completely dehydrogenated pentenoanthracenes (XII, R = C_9H_{19} and C_8H_{17}),[1,2] have been examined biologically by Dr. Murray J. Shear of the National Cancer Institute[4] but no evidence of carcinogenic potency could be detected. It is known, however, that large alkyl residues diminish or abolish carcinogenicity[5] and, consequently, the negative results with the compounds bearing a C_8- or C_9-side chain do not exclude the possibility that carcinogenicity may be found among anthrasteroids with a small side chain or none at all. In fact, the most thoroughly investigated cases of the relationship of steroids to cancer deal with the hormones, and it is of interest that these materials bear a side chain with no more than two carbon atoms. We have accordingly undertaken the synthesis of several anthrasteroids which are related to the hormones and wish to report here the conversion of pregnenolone (I, R = $COCH_3$) to an anthrasteroidal analog of progesterone, *viz.*, 5,7,9,14-anthrapregnatetraen-20-one (XI, R = $COCH_3$).[6]

The synthesis of XI from I (R = $COCH_3$) was accomplished through the series of reactions used previously for the conversion of other steroids to anthrasteroids. The steps are: (a) introduction of a 5,7-diene system (II to IV) and then a 5,7,9(11)-triene system (IV to VI),[1] (b) acid-catalyzed dehydration to a 3,5,7,9(11)-tetraene (VI to VIII) which apparently proceeds by an allylic mechanism involving prior double bond migration (VI to VII),[7a] (c) acid-catalyzed double bond migration which converts VIII to the 5,7,9(11),14-tetraene X through the probable intermediate tetraene IX,[7a,b] and, finally, (d) acid-catalyzed rearrangement and aromatization of X to the anthrasteroid XI.[7b] In the present case, the conversions b, c and d, *i.e.*, VI→XI, were carried out in one experimental step in a 20% over-all yield of isolated, crystalline material. The prerequisite conversion of I to IV and then to VI was carried out in the manner we have previously reported for the preparation of 5,7,9(11)-cholestatrien-3β-ol from cholesterol (VI from I, R = C_8H_{17}) using the isocaproate technique.[1] The yield of the pure, crystalline 7-dehydro compound IV (R = $COCH_3$, R′ = $CO(CH_2)_2CH(CH_3)_2$) from pregnenolone isocaproate II (R = $COCH_3$, R′ = $CO(CH_2)_2CH(CH_3)_2$) was 34% which represents approximately a 100% increase in the yield of IV compared to the yields reported earlier for the benzoate[8a] or acetate.[8b] The advantage of the iso



(1) Part IV, W. R. Nes, R. B. Kostic and E. Mosettig, THIS JOURNAL, **78**, 436, 6423 (1956).

(2) (a) W. R. Nes and E. Mosettig, *ibid.*, **75**, 2787 (1953); (b) W. R. Nes and E. Mosettig, *ibid.*, **76**, 3182 (1954).

(3) (a) A. Haddow, *Ann. Rev. Biochem.*, **24**, 696 (1955); (b) A. Butenandt and H. Dannenberg, *Handbuch Allgem. Pathol.*, **6** (Part 3), 150 ff. (1956); (c) H. H. Inhoffen, *Naturwissenschaften*, **40**, 455 (1953); (d) H. H. Inhoffen and K. Brückner, *Fortschr. Chem. org. Naturstoffe*, **11**, 116 (1954); (e) H. Dannenberg, *Krebsforschung und Krebsbekämpfung*, **2**, 36 (1957).

(4) The compounds were dissolved in mineral oil (Nujol) in a concentration of 10 mg./ml. A single injection of 2 mg. was given, subcutaneously in the left axilla, to each of 50 young adult CAF_1 mice equally divided between the sexes. The biological experiments were begun between October, 1953, and March, 1954. Periodic examination of the mice revealed only a single spontaneous carcinoma, but no induced tumors were obtained by the time the experiments were ended after a period of about two years.

(5) (a) C. M. Badger, *Brit. J. Cancer*, **2**, 309 (1948); (b) J. L. Hartwell, "Survey of Compounds which have been Tested for Carcinogenic Activity," Vol. I, U. S. Public Health Service Publication No. 149, 1951, pp. 128, 129, 299; (c) H. Dannenberg, *Z. Krebsforsch.*, **62**, 220 (1957).

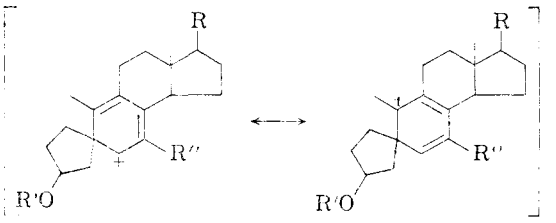
(6) The preparation of an anthrasteroid related to the androgens and estrogens is the subject of Part VI, W. R. Nes, J. A. Steele and E. Mosettig, THIS JOURNAL, **80**, 5233 (1958); see also ref. 1.

(7) (a) W. R. Nes and J. A. Steele, *J. Org. Chem.*, **22**, 1157 (1957); (b) W. R. Nes, THIS JOURNAL, **78**, 193 (1956).

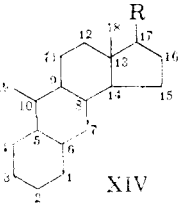
(8) (a) C. Djerassi, J. Romo and G. Rosenkranz, *J. Org. Chem.*, **16**, 754 (1951); (b) R. Antonucci, S. Bernstein, D. Giancola and K. J. Sax, *ibid.*, **16**, 1126 (1951).

caproate method for the preparation of pure 7-dehydrosteroids probably results from steric inhibition of elimination at C-4 caused by the bulky ester group as well as an improved solubility relationship between the double bond isomers which are formed.[9] The introduction of the third double bond at C-9(11) was effected with mercuric acetate by a modification of the procedures already reported in the literature.[8a,10] The isolation of pure VI was best accomplished after hydrolysis of the ester. The yield of ester VI (R = $COCH_3$, R′ = $(CH_3)_2$-$CH(CH_2)_2CO$) as the crude chromatographed product was 50%. It showed only the absorption of the 5,7,9(11)-triene system, but it failed to crystallize. Similarly, the acetate of IV (R = $COCH_3$) on conversion to the acetate of VI (R = $COCH_3$) with mercuric acetate failed to give the correct melting point except on extensive recrystallization. An explanation for this may be that VI was contaminated with a small amount of the 3-epimer.[11] The crystalline alcohol VI (R = $COCH_3$, R′ = H) could be obtained after hydrolysis in an over-all yield of 44% (crude) and 26% (pure) from the isocaproate of IV (R = $COCH_3$). Hydrolysis was employed principally as a means for facilitating the final isolation of the anthrasteroid XI (R = $COCH_3$). The main by-products of the conversion of VI to XI retain an ester or hydroxyl group depending on whether an ester or free alcohol is used as starting material (VI). Since XI (R = $COCH_3$) is a ketone, it is separated readily from hydroxylated by-products by chromatography but less readily separated from the corresponding esters.

The anthrasteroid XI (R = $COCH_3$) prepared in this investigation can be assumed with certainty to have the structure as shown in the formula. Its spectroscopic properties in the infrared (λ_{max} 5.84 and 12.27 μ) and ultraviolet (λ_{max} 221, 226, 266, 296, and 308 mμ) regions are exactly those qualitatively and quantitatively to be expected[2,12] for this type of compound, and the structural uncertainties which remained following our earlier work[2] have been removed by Burgstahler[13] whose degradation of 5,7,9,14-anthracholestatetraene[1] led to 3,9-dimethylanthracene which proved the position of the methyl group on the aromatic ring and the position of the conjugated double bond. Since there is no reason to believe that the stereochemistry at the only two asymmetric centers (C-13 and C-17) in XI is different from that present in the steroids, the problem of structure is now completed for the anthrasteroids prepared by acid-catalyzed dehydration, rearrangement and aromatization of 3β-hydroxy-5,7,9(11)-trienes and their esters. There is, however, one point which requires further study for anthrasteroids prepared by the dienone–phenol rearrangement of 7-keto-5,8-dienes[14] and the photodehydrogenation and rearrangement of provitamin-D.[15] In these two cases the conversion does not remove the substituent in ring A. If the mechanism of rearrangement involves a spiro intermediate XIII, then the position of the substituent (originally at C-3) in the final product is uncertain, because, as Bladon[14a] has pointed out, the spiro form XIII can rearrange to an anthrasteroid by the migration of either of two carbon atoms (C-1 or C-4) to C-6. This problem is obviated for the conversions VI → XI which proceed with initial dehydration at C-3, for, unless there is some additional substituent in ring A, the spiro intermediate will lack substitution and be symmetrical. The question of the mechanism[16] bears also on nomenclature.[17] Bladon[14a] has used a numbering for anthrasteroids as shown in XIV. Such a system suggests that C-1 is finally attached to C-6,[3d] but this remains to be established. Nevertheless, the numbering shown in XIV has great merit in that, as we now know,[2,7,13] it correctly identifies all of the carbon atoms in rings B, C and D with the original steroid. We are adopting



XIII, R'' = H or OH



XIV

(9) Improved yields of 7-dehydrosteroids have also been claimed in several recent patents by the use of dehydrobrominating agents other than collidine. Thus, ammonia (A. Wander, British Patent 760,460, Oct. 31, 1956; *C. A.*, **51**, 9722 (1957)) and sodium silicate (H. C. Klein and R. Kapp, U. S. Patent 2,776,304, Jan. 1, 1957; *C. A.*, **51**, 7447 (1957)) are reported to give 7-dehydrocholesterol in a yield of *ca.* 50%. Both of these were tried with pregnenolone acetate but failed to give any isolable pure 7-dehydro derivative. The use of quinaldine has been reported (K. H. Schaaf, U. S. Patent 2,546,788, Mar. 27, 1951, and W. L. Ruigh and D. H. Gould, U. S. Patent 2,546,787, Mar. 27 1951) to improve the preparation of 7-dehydrocholesterol, but the experiments detailed do not give any yields of isolated pure steroid.

(10) R. Antonucci, S. Bernstein, D. Giancola and K. J. Sax, *J. Org. Chem.*, **16**, 1159 (1951).

(11) Assuming an analogy between the reaction of mercuric acetate with IV and its reaction with 6,8-cholestadien-3β-ol *p*-nitrobenzoate (D. H. R. Barton and W. J. Rosenfelder, *J. Chem. Soc.*, 2381 (1951)), we believe that the 5α-acetoxy-6,8-diene V may be an intermediate in the conversion of IV to VI. We have actually observed the presence of a by-product absorbing at 286 mμ which is close to the expected maximum for such a homoannular diene. If V were the intermediate, an acid-catalyzed elimination reaction at C-3 with neighboring group participation from the 5α-acetoxy group would lead to the 3-epimer of VI. Such a reaction already has been observed (D. C. Burke, J. H. Turnbull and W. Wilson, *J. Chem. Soc.*, 3237 (1953)).

(12) L. Scheer, W. R. Nes and P. Smeltzer, THIS JOURNAL, **77**, 3300 (1955).

(13) A. W. Burgstahler, *ibid.*, **79**, 6017 (1957).

(14) (a) P. Bladon, *J. Chem. Soc.*, 2176 (1955); (b) K. Tsuda, K. Arima and R. Hayatsu, THIS JOURNAL, **76**, 2933 (1954).

(15) K. Tsuda and R. Hayatsu, *ibid.*, **77**, 3089 (1955).

(16) Burgstahler's proof[13] of the position of the methyl group in anthrasteroids establishes that of the two possible sites for the initial carbon–carbon rupture[2b] the one involving a break between C-1 and C-10 is correct. It is interesting to note that this means that the migration of a carbon atom from C-10 is not stereospecific, because we have already shown that the same anthrasteroid is obtained by acid-catalyzed dehydration and rearrangement regardless of the configuration at C-10.[2b] Bladon[14a] has observed the same non-stereospecificity in the dienone–phenol rearrangement leading to anthrasteroids.

(17) For our proposal concerning the nomenclature of the parent carbon skeletons of anthrasteroids see ref. 2d, footnote 6.

this system realizing that it may or may not correctly identify the carbon atoms of ring A.

The anthrasteroid XI (R = $COCH_3$) is being examined for carcinogenic activity by Prof. N. P. Buu-Hoi of the Institut du Radium in Paris. Compounds XI (R = $COCH_3$), VI (R = $COCH_3$, R′ = H) and IV (R = $COCH_3$, R′ = H) were tested for hormonal behavior by the Endocrinology Branch of the National Cancer Institute under the direction of Dr. Roy Hertz. None showed any potency in the Clauberg test in the rabbit at ten times the effective dose of progesterone. They were also examined for potential anti-progestational effects at comparable levels against half-maximal doses of progesterone and were found to be ineffective.

Experimental[18,19]

5-Pregnen-3β-ol-20-one Isocaproate (II from I).—Pregnenolone was esterified with 4-methyl-pentanoyl chloride in pyridine in the usual manner. The colorless ester formed in elongated, flat prisms, m.p. 100–102°, $[\alpha]D$ +19°.

Anal. Calcd. for $C_{27}H_{42}O_3$ (414.6): C, 78.21; H, 10.21. Found: C, 78.40; H, 10.25.

5,7-Pregnadien-3β-ol-20-one Isocaproate (IV from II).—The isocaproate (50 g.) of 5-pregnen-3β-ol-20-one was dissolved in 200 ml. of carbon tetrachloride. To this was added 800 ml. of petroleum ether (b.p. 60–71°) and 28.8 g. of N-bromosuccinimide. The mixture was heated to boiling under reflux; the heating was then discontinued and the mixture was irradiated with two General Electric "photospot" lamps (No. RSP2) held *ca.* 1 cm. from the flask walls. Irradiation with spontaneous reflux was maintained for 1.0 hr. The mixture was cooled in ice and filtered. The precipitate of succinimide and excess N-bromosuccinimide after drying weighed 15.9 g. The filtrate was evaporated to dryness under reduced pressure in a bath at 40°. The crude **7-bromo-5-pregnen-3β-ol-20-one isocaproate** (III) was not further purified. It was dissolved in 150 ml. of xylene and added dropwise to a well stirred, refluxing mixture of 40 ml. of collidine in 350 ml. of xylene. The addition required 50 min. after which the mixture was refluxed an additional 60 min., cooled in ice, and filtered. The dried collidine hydrobromide which was collected weighed 24.4 g. (100% based on pregnenolone isocaproate). The filtrate was evaporated to dryness under reduced pressure. The residue was dissolved in 130 ml. of hot methanol and allowed to cool slowly to −20°. The resulting crystals (mixed with some gum) were recrystallized once further from 130 ml. of methanol (cooling to 0°) and three times from 130 ml. of methanol and sufficient water to induce crystallization while hot (cooling to 0°). This procedure consistently gave 17 g. (34%) of pure 7-dehydropregnenolone isocaproate (IV) as essentially colorless long flat prisms, m.p. 105–108°, $[\alpha]D$ −19°; λ_{max} 271.5, 282.5 and 294.7 mμ (ε 11250, 12000 and 7000); λ_{infl} 262 mμ (ε 7700), λ_{min} 232 mμ (ε 1300). A *ca.* 40% yield of material of somewhat less purity was obtained by carrying out fewer recrystallizations.

(18) All melting points were determined on a Kofler block and are recorded as read. Analyses are by the Microanalytical Service Laboratory of this institute under the direction of Dr. William C. Alford. Rotations were taken in 1% chloroform solutions at 20° by Mrs. E. Peake. The infrared spectra were determined on a Perkin-Elmer double beam spectrophotometer (model 21) in CS_2 by H. K. Miller. Ultraviolet spectra were determined on a Cary recording spectrophotometer (model 11) with the assistance of Mrs. C. I. Wright. The ultraviolet spectra were measured in an ethanol solution unless otherwise noted. The ultraviolet absorption from 220 to 400 mμ of every fraction from chromatograms was determined and was the basis for combinations of fractions as well as for the evaluation of the constituents present. The elutions with a given solvent were routinely continued until the material in question no longer was being removed at a significant rate. The alumina used was purchased from M-Woelm-Eschwege, activity grade 1, and was of an acidic or basic nature as indicated. Solvents were purified, distilled and dried with the help of J. Lyons excepting ethanol and chloroform which were the usual commercial grades of reagent material.

(19) In the formulas referred to in this section, R = $COCH_3$.

Anal. Calcd. for $C_{27}H_{40}O_3$ (412.6): C, 78.59; H, 9.77. Found: C, 78.50; H, 9.78.

The procedure described above was successful with batches varying from 25 to 75 g.

5,7-Pregnadien-3β-ol-20-one.—7-Dehydropregnenolone isocaproate was hydrolyzed with potassium carbonate in methanol containing a little water. The product was recrystallized from chloroform–ethanol and gave large, colorless prisms, m.p. 220–223°, $[\alpha]D$ −77°; λ_{max} 271, 282 and 294 mμ (ε 11200, 12000 and 7000), λ_{infl} 263 mμ (ε 7700); lit. m.p. 216–220°, $[\alpha]D$ −67°,[8a] and m.p. 228–230°, $[\alpha]D$ −79°.[8b]

5,7,9(11)-Pregnatrien-3β-ol-20-one (VI).—To a solution of 15 g. of 7-dehydropregnenolone isocaproate (IV) in 366 ml. of chloroform was added a solution of 30 g. of mercuric acetate in 550 ml. of acetic acid and the solution was allowed to remain at room temperature (24°) for 40 hours. Mercurous acetate began to precipitate immediately and at the end of the reaction was collected by filtration and dried. It weighed 21.8 g. (115% of two molecular equivalents of starting steroid). When the reaction was carried out for less time the yield of mercurous acetate was less, but the product was contaminated with starting material as shown by ultraviolet assay. The filtered reaction mixture was diluted with chloroform and washed with water. The organic layer was washed with aqueous potassium carbonate until it was neutral and finally with water. Removal of the solvent under reduced pressure left a reddish oil (λ_{max} 286, 324 and 338 mμ; ε 10500, 6800 and 4400) which was dissolved in a small amount of ether and adsorbed on 280 g. of alumina ("basic"). Elution with 600 ml. of ether in several fractions yielded **5,7,9(11)-pregnatrien-3β-ol-20-one isocaproate** as a light yellow oil showing only the characteristic ultraviolet spectrum of the 5,7,9(11)-triene system. It weighed 7.5 g. (50%), but crystallized only with difficulty and was therefore not purified further. It was dissolved in 116 ml. of methanol under reflux and to this was added 39 ml. of hot water containing 6.5 g. of potassium carbonate. After about 15 minutes a precipitate began to form. After a total of two hours the solution was diluted with water, cooled to 0°, and filtered. The resulting 5,7,9(11)-pregnatrien-3β-ol-20-one was washed well with water and with a little cold methanol. The dried product weighed 5.05 g. (44%) and melted at 188–192°, λ_{max} 324 mμ (ε 10600), λ_{infl} 312 and 338 mμ (ε 9400 and 6900). A portion of this was recrystallized twice from benzene yielding 3.0 g. of the pure alcohol as colorless prisms, m.p. 204–207°, $[\alpha]D$ +271°, λ_{max} 324 mμ (ε 11000), λ_{infl} 313 and 338 mμ (ε 9800 and 6900), λ_{min} 247 mμ (ε 480); lit. m.p. 201–203°, $[\alpha]D$ +285°,[8a] and m.p. 221–225°, $[\alpha]D$ +304°.[10]

5,7,9,14-Anthrapregnatetraen-20-one (XI from VI).—A solution of 5.0 g. of 5,7,9(11)-pregnatrien-3β-ol-20-one in 300 ml. of 0.079 *M* HCl in chloroform was allowed to remain at room temperature (24°) for 2.0 hr. during which time the solution turned dark red. It was extracted with an aqueous potassium carbonate solution which caused the color to turn to light yellow. Removal of the solvent under reduced pressure left an oil which was chromatographed on 150 g. of alumina ("basic"). The column was eluted with ether. The fractions containing the anthrasteroid (2.2 g. based on the intensity of the maximum at 266 mμ) were contaminated with an impurity absorbing near 340 and 352 mμ. The intensity of absorption at 340 mμ was 17% of the intensity of the maximum at 266 mμ for the anthrasteroid and this relative intensity was unchanged over 27 fractions of 22 ml. each. Twelve fractions under the principal part of the elution curve were combined and evaporated to dryness, yielding 2.1 g. of an oil which was crystallized twice from ethanol–water. The resulting anthrasteroid weighed 0.93 g. (20%) and melted at 130–133°. Further recrystallization from ethanol–water gave the analytical sample as colorless needles, m.p. 138–139°, $[\alpha]D$ −33°; $\lambda_{max}^{isooctane}$ 221, 226, 266, 296, and 308 mμ (ε 24600, 25300, 16300, 2400 and 1900); $\lambda_{infl}^{isooctane}$ 233 mμ (ε 16300) λ_{max} 5.84 and 12.27 μ.

Anal. Calcd. for $C_{21}H_{26}O$ (294.4): C, 85.66; H, 8.90. Found: C, 85.23; H, 8.94.

The anthrasteroid XI was obtained in essentially the same yield from either crude (m.p. 188–192°) or pure (m.p. 204–207°) 5,7,9(11)-pregnatrien-3β-ol-20-one (VI).

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