Anal. Calcd. for $C_{13}H_{14}O_6N_2$: C, 53.06; H, 4.80; N, 9.52. Found: C, 53.24; H, 4.94; N, 9.65.

Catalytic Hydrogenation of 2-Methylcyclopentanone.—2-Methylcyclopentanone (5.3 g.) was hydrogenated in the presence of 1.0 g. of platinum oxide catalyst. After the absorption of hydrogen had ceased, the catalyst was filtered and the concentrated filtrate was treated with 14.6 g. of 3,5-dinitrobenzoyl chloride in 35 ml. of dry pyridine (twenty-four hours, room temperature). The reaction mixture was then diluted with water and extracted with ether and a small amount of methylene chloride. The extracts were combined and washed successively with water, dilute hydrochloric acid, water, dilute sodium hydroxide solution and saturated sodium chloride, filtered through anhydrous sodium sulfate and concentrated to dryness. The residue was fractionally crystallized from methanol and gave small amounts of two pure products, A, m. p. 66–67°, and B, m. p. 82–84°. Mixed melting point determinations with VIII and IX, respectively, showed no depression.

Reaction of Cyclopentene Oxide with Methylmagnesium Iodide.—Cyclopentene oxide[16] (3.90 g.) was added slowly to an ethereal solution of 2 molar equivalents of methylmagnesium iodide. After standing overnight at room temperature in an atmosphere of dry nitrogen, the reaction mixture was acidified with dilute hydrochloric acid. The aqueous phase was repeatedly extracted with ether, and the combined extracts were washed with saturated sodium chloride solution containing a little sodium bisulfite to remove traces of iodine. The 3,5-dinitrobenzoate was prepared by the usual procedure and after purification by recrystallization from ethanol, in which it was rather insoluble, a pure sample of cyclopentene iodohydrin 3,5-dinitrobenzoate melting at 117–118° was obtained.

Anal. Calcd. for $C_{12}H_{11}O_6N_2I$: C, 35.48; H, 2.73; N, 6.90; I, 31.25. Found: C, 35.24; H, 2.80; N, 6.82; I, 30.97.

Reaction of Cyclopentene Oxide with Methyllithium.—Cyclopentene oxide (2.00 g.) was added to a solution of 1.2 molar equivalents of methyllithium in ether. The reaction mixture was refluxed under an atmosphere of nitrogen for one hour, and the ether was evaporated in a stream of nitrogen. After the residue had been heated on the steam-bath for two hours, a small amount of water was added, and the product taken into ether. The residue obtained after removal of the solvent afforded 3.60 g. (51%) of a dinitrobenzoate melting at 78–81°. Two recrystallizations from methanol gave a sample, m. p. 82–83.5°, that did not depress the melting point of IX or of B, described above.

Summary

The reactions of *cis*- and *trans*-1-acetyl-2-methylcyclohexane with perbenzoic acid yield, respectively, *cis*- and *trans*-2-methylcyclohexanyl acetate, identified by saponification and conversion into the corresponding acid phthalates. Analogous oxidations of *cis*- and *trans*-1-acetyl-2-methylcyclopentane similarly yield *cis*- and *trans*-2-methylcyclopentanyl acetate, respectively. The latter products were saponified and converted into 3,5-dinitrobenzoates, the structures of which were established by independent syntheses. These products are not identical with the 3,5-dinitrobenzoates of "*cis*-" and "*trans*"-2-methylcyclopentanol reported by Hückel and Kindler.

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[CONTRIBUTION FROM THE SLOAN-KETTERING INSTITUTE FOR CANCER RESEARCH]

Perbenzoic Acid Oxidation of 20-Ketosteroids and the Stereochemistry of C-17[1]

BY T. F. GALLAGHER AND THEODORE H. KRITCHEVSKY

The oxidation of 20-ketosteroids to acetoxy derivatives of C_{19} steroids by means of persulfuric acid has been described by Marker[2]; Burckhardt and Reichstein[3] and Sarett[4] have examined the same reaction with perbenzoic acid as the oxidizing agent. These authors studied the reaction only with 20-keto derivatives having the normal or 17β orientation of the side chain and noted that only a single diastereoisomer was isolated from the reaction. In order to provide more complete information about the stereochemical course of the reaction and to gain an insight into the reaction mechanism, we have investigated the oxidation of 3α-acetoxy-17α-pregnan-20-one where the side chain is attached in the opposite configuration to that studied in the previous investigations. At the same time Dr. Richard B. Turner at Harvard University undertook an investigation of this reaction upon simpler diastereoisomers.[5] The results of both investigations leave little doubt of the course and mechanism of the reaction. It is shown that the oxidation proceeds without inversion of configuration at C-17 and as a result the procedure can be useful for the determination of configuration as well as a valuable preparative tool. The reactions are summarized in Fig. 1.

(1) This investigation was supported by grants from the Jane Coffin Childs Memorial Fund for Medical Research, the Anna Fuller Fund, the Lillia Babbit Hyde Foundation, and the National Cancer Institute, United States Public Health Service.

(2) Marker, THIS JOURNAL, **62**, 2543 (1940).

(3) Burckhardt and Reichstein, *Helv. Chim. Acta*, **25**, 1434 (1942).

(4) Sarett, THIS JOURNAL, **69**, 2899 (1947).

Experimental

3α,17β-Etiocholanediol from 3α-Acetoxypregnan-20-one.—A solution containing 230 mg. of 3α-acetoxypregnan-20-one and 88.5 mg. of perbenzoic acid in 1.1 ml. of chloroform was stored at room temperature for seven days. The neutral fraction was isolated in the usual manner and fractionated with the Girard reagent T. 95 mg. of ketonic material and 127 mg. of non-ketonic product were obtained. The non-ketonic fraction was sublimed in high vacuum and yielded 100 mg. of sublimate. 10 mg. of crystalline product, m. p. 215–220°, was removed and the remainder was saponified with 0.25 *N* sodium hydroxide in 50% alcohol at 60° for one-half hour. The crystalline product upon neutralization was recrystallized from ethanol and yielded 69 mg. of 3α,17β-etiocholanediol, m. p. 229–232°; $[\alpha]_D$ +23° (ethanol). One recrystallization gave the pure product which melts 236–236.5°;

(5) Turner, *ibid.*, **72**, 878 (1950).

$[\alpha]$D +25° (ethanol); reported[6] constants are m. p. 236–236.5°; $[\alpha]^{21}$D +24.8° (ethanol). No other compound could be isolated.

The ketonic fraction after room temperature hydrolysis yielded 3α-hydroxypregnan-20-one, m. p. 142–145°; $[\alpha]$D +113° ($CHCl_3$) identical in all respects with the known compound.

3α,17α-Etiocholanediol and 17α-Acetoxyetiocholan-3α-ol.—A solution containing 178 mg. of 3α-acetoxy-17α-pregnan-20-one, m. p. 158.5–160°, $[\alpha]$D −30.6° (ethanol), and 95 mg. of perbenzoic acid in 0.75 ml. of chloroform was stored at room temperature for eight days when all of the perbenzoic acid had been consumed; 216 mg. was isolated in the usual manner and separated with the Girard reagent T. The non-ketonic fraction weighed 97 mg. and the ketonic fraction weighed 80 mg. The non-ketonic fraction was acetylated with acetic anhydride in the presence of perchloric acid and despite chromatography, no crystalline product was obtained. The oily fractions were combined and after hydrolysis at room temperature with 0.25 *N* sodium hydroxide in 50% ethanol for one half hour, 78 mg. of product was obtained. Chromatography upon a mixture of magnesium silicate and Celite yielded 53 mg. in the eluates obtained with 1:19 ether–benzene and 12 mg. of crystalline material eluted with 2:5 ether–benzene.

The 12 mg. of crystalline material was recrystallized from acetone–90° petroleum ether and in two crops yielded 10 mg. of pure 3α,17α-etiocholanediol, m. p. 226.5–227°; $[\alpha]^{28}$D 0° (ethanol). Schneider and Mason[7] have described this substance as 3α,17β-etiocholanediol with m. p. 227–228°; $[\alpha]^{28}$D 0° (ethanol).

The 53 mg. of oily material was crystallized from 90° petroleum ether and melted 72–74° after slight sintering at 70°. Recrystallization from the same solvent yielded 17α-acetoxyetiocholan-3α-ol which melted 75–76°, clear at 78°, after slight sintering at 70°. The melting behavior was not altered upon further recrystallization; $[\alpha]^{26}$D 0° (chloroform). *Anal.* Calcd. for $C_{21}H_{34}O_3$: C, 75.40; H, 10.24. Found: C, 75.63; H, 10.07. All the crystalline products and mother liquors were combined and hydrolyzed in 20 ml. of 0.5 *N* sodium hydroxide under reflux for thirty-five minutes. After cooling, extraction with ethyl acetate and washing with sodium chloride solution, removal of the solvent yielded a crystalline residue. This was recrystallized from acetone and 3α,17α-etiocholanediol, m. p. 227–228.5° was obtained. The product gave no depression of melting point when admixed with the sample of the diol previously obtained in the chromatogram. No other product was isolated from the reaction.

Discussion

It is clear from our results that the configuration of the product obtained from the perbenzoic acid oxidation of a 20-ketosteroid is determined by the initial configuration of the C-17 acetyl group. Thus a 17β-20-ketone yielded a 17β-acetoxy derivative while the 17α-acetoxy compound was obtained from a 17α-20-ketosteroid. It could be concluded from these results that the reaction proceded without effective inversion of configuration. The validity of the conclusion then depends upon the certainty with which the configuration at C-17 can be established for 20-keto and 17-hydroxy steroids.

The stereochemistry of C-17 of the steroid nucleus has received extensive study since the orientation of substituent groups exerts such marked influence on biological action and chemical reactivity. Steroids of animal origin with a carbon

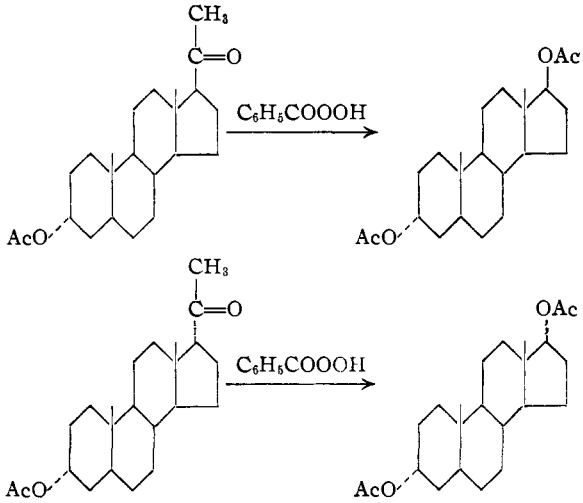

Fig. 1.

side chain at C-17 such as cholesterol and its biochemically significant derivatives, the bile acids, progesterone and the hormones of the adrenal cortex, all have the common feature that the substituent at C-17 is attached to the nucleus in the same spatial relationship. It has been demonstrated with considerable certainty from both physical and chemical evidence that this configuration is β, *i. e.*, *cis* to the angular methyl groups C-18 and C-19.[8,9]

The spatial relations of the 17-hydroxyl group in the C_{19} steroids have not been studied by such critical methods. The 17-hydroxyl group of testosterone was originally designated "*trans*" to the C-18 methyl by Ruzicka, Furter and Goldberg,[10] who arrived at this conclusion from the application of the Auwers–Skita rule that catalytic reduction in neutral solution leads to a preponderance of the *trans* isomer. Moreover "*trans*" esters at C-17 were saponified more readily than their epimers and this was consistent with an extension of the principle of Vavon and Jakubowicz[11] that esters of *cis* substituents were more difficultly saponified than *trans*. Since two independent methods led to the same conclusion, the orientation of the 17-hydroxyl group seemed established and for many years, the α configuration for testosterone and estradiol was widely accepted as evidenced by the common names "α-estradiol" and "*cis* testosterone" for the respective natural and unnatural configuration at C-17.

Many facts, however, were difficult to reconcile with a 17α-hydroxyl group in testosterone and related compounds. Goldberg, *et al.*,[12] have summarized certain of these and have called attention

(6) Ruzicka, Goldberg and Bosshard, *Helv. Chim. Acta*, **20**, 541 (1937).

(7) Schneider and Mason, *J. Biol. Chem.*, **175**, 231 (1948).

(8) Carlisle and Crowfoot, *Proc. Roy. Soc.* (*London*), **A184**, 64 (1945).

(9) Sorkin and Reichstein, *Helv. Chim. Acta*, **29**, 1218 (1946).

(10) Ruzicka, Furter and Goldberg, *ibid.*, **21**, 498 (1938).

(11) Vavon and Jakubowicz, *Bull. soc. chim. France*, [4] **53**, 581 (1933).

(12) Goldberg, Sice, Robert and Plattner, *Helv. Chim. Acta*, **30**, 1441 (1937).

to the marked similarity of the C-17 and C-12 hydroxyl groups in molecular models. Although these authors were primarily concerned with D-homosteroids, the C-12 and C-17 hydroxyls in the normal steroids with 5-membered ring D *in its usual closure* seem equally comparable since each position is subject to almost identical steric influence. The configuration at C-12 in the bile acids is known with considerable certainty[9,13] and esters of 12β-hydroxy derivatives have been shown to be more easily saponified than the α isomers.[14] It would be expected, then, that C-17 esters of the α configuration would be more difficultly saponified than the β oriented derivatives. Since esters of testosterone are more readily hydrolyzed with base than the C-17 epimer,[10] testosterone is probably a 17β-hydroxy steroid.

A somewhat analogous argument relates the position of the 17-hydroxyl to the known orientation of a carboxyl group attached to C-17.[15,16] The velocity of alkaline saponification of epimeric 17-acetoxy derivatives has been compared qualitatively with esters of the etio acids and it was observed that the β configuration at 17 is associated with greater ease of saponification of the ester group. While this evidence is not unobjectionable since the attack of hydroxyl ion at the carbonyl in one instance is immediately adjacent to the asymmetric center while in the 17-acetoxy compound, it is farther removed, the results are consistent with those obtained in other ways.

From this discussion it is apparent that a variety of indicative evidence is in agreement on the orientation of substituents at C-17. The conclusion presented previously, that the per-acid oxidation of a 20-ketosteroid is accomplished without inversion, is therefore permissible. When the results of Turner are considered with this report, it is apparent that the configuration of alcohols at C-17 can be established unequivocally by this reaction. Apart from the obvious necessity for reversal of the previously assigned configurations, which has been thoroughly treated by Fieser and Fieser[17] in their excellent monograph, certain other consequences of these results are of considerable interest for the stereochemistry of steroids.

Fieser and Fieser[18] have considered the reactions of C-17 and have concluded that the attack of carbon proceeds more readily from the α or rear face while the front of the molecule has more space available for the substituent group. This generalization can be more inclusively stated *that when there is a plane of symmetry at C-17 the entering group always attaches to C-17 in the α configuration.* A survey of the known reactions at C-17 demonstrates without exception the validity of this "rule of the rear." Thus catalytic reduction, $LiAlH_4$, R-Mg-Br, K—C≡C—H, OsO_4, C_6H_5-COOOH and the like all result in the attachment of the *entering group* preponderantly in the α configuration of C-17. The major component of any reaction at C-17 will then represent the product where the entering group has assumed the α position and with a rapid reaction very little of the alternate isomer will be present. On the other hand where an equilibrium is possible a minor though sensible proportion of the product may represent attack of C-17 from the β face. The isomeric mixture achieved when 20-ketosteroids or esters of the etio acids are equilibrated with acid or alkali conforms to the rule since the entering group—the proton—assumes predominantly the α configuration of C-17. The ring D ketols investigated by Huffman and Lott[19] are in conformity with the rule since the rearrangement product has a 17β-hydroxyl group resulting from the attachment of a proton to C-17 from the α face of the carbon atom. The Serini reaction on the other hand involves an asymmetric intermediate and the direction of the attachment of the proton to C-17 is governed by the orientation of the other substituents.[20]

The similarity of reactions at C-17 and C-12 has been noted earlier in the discussion. The catalytic reduction of a 12-keto group is so greatly influenced by the experimental conditions that the configuration of the product can hardly be used as an index of hindrance effects; with a platinum catalyst in glacial acetic acid solution the 12α-hydroxyl group appears to be the sole product[14] whereas Raney nickel in alkaline solution results in the formation of a 12β-hydroxyl.[21] Reduction of a C-12 carbonyl group with $LiAlH_4$ might provide more certain stereochemical evidence. The parallelism of C-12 and C-17 in other reactions is indeed striking and this resemblance can be used to explain some hitherto puzzling experimental results at C-12. It is known that when a Ring C ketol is equilibrated with base the principal product is an 11-keto-12β-hydroxy derivative.[22] The formation of the 12β-hydroxyl group in this ketol through an intermediary enediol had been considered an exceptional stereochemical result since the configuration at C-12 is the opposite of that obtained when an 11-ketone is brominated. It can be shown, however, that both reactions take a clearly predictable course. Just as with C-17, the entering group, in this instance the proton, attaches to C-12 from the rear and the β-hydroxyl group therefore predominates in the product. Since an equilibrium is possible, a minor proportion of the

(13) Gallagher and Long, *J. Biol. Chem.*, **162**, 495 (1946).
(14) Koechlin and Reichstein, *Helv. Chim. Acta*, **25**, 935 (1942).
(15) Von Euw and Reichstein, *ibid.*, **30**, 205 (1947).
(16) Heusser, Meier and Ruzicka, *ibid.*, **29**, 1250 (1946).
(17) Fieser and Fieser, "Natural Products Related to Phenanthrene," third edition, New York, 1949. Note however that while estriol is correctly formulated by Fieser and Fieser (p. 316), Huffman and Lott[19] have adhered to the older and incorrect configuration at C-17. As a consequence all of their configurations must be revised and their conclusions about the structure of triols prepared by other investigators are clearly in error.
(18) Fieser and Fieser, *Experientia*, **4**, 285 (1948).
(19) Huffman and Lott, THIS JOURNAL, **71**, 719 (1949).
(20) Shoppee, *Experientia*, **4**, 418 (1948).
(21) Wenner and Reichstein, *Helv. Chim. Acta*, **26**, 965 (1944).
(22) Borgstrom and Gallagher, *J. Biol. Chem.*, **177**, 951 (1949).

12α-hydroxy compound is also found. The result is thus readily explicable and in complete agreement with the "rule of the rear." When methyl 3α-acetoxy-11-ketocholanate is brominated, the entering group must also approach the enol extending to C-12 from the rear and as a consequence the product obtained should be the 12α-bromo derivative which is in agreement with the experimental fact.[23]

When an ethylenic group between C-11 and C-12 is treated with halogen,[24] hypobromous acid[25] or active oxygen[13,26,27] the entering group becomes attached to C-12 in the α configuration. With methyl 3α-acetoxy-Δ^{11}-cholenate the products are respectively the 11β,12α-dibromo, the 11β-hydroxy-12α-bromo and 11α,12α-epoxy derivatives. These three reactions illustrate an important corollary of the "rule of the rear" since they demonstrate that the configuration of C-11 in the product is determined by the orientation of the substituent at C-12. Halogenation of an ethylene proceeds by a reaction mechanism which results in *trans* addition. The attack of C-12 at the α face therefore precedes that of C-11 and is the stereochemically determining step in the reaction. The halogen, as a consequence, is forced to assume the greatly hindered β configuration at C-11. The result is in sharp contrast to that obtained when bromine is substituted at C-11 in the halogenation of a C-12 ketone. Here the enol is not subjected to the directing influence of a substituent at C-12 and the product obtained is almost exclusively the 11α-bromo derivative resulting from attack of the unhindered rear face of C-11.

The *trans* addition of hypobromous acid to the 11,12-ethylene is another instance of the influence which the configuration at C-12 exerts on the orientation of a substituent at C-11. The formation of an epoxide from C-11 to C-12 with perbenzoic acid is especially interesting, since, unlike the previous examples, the reaction must proceed by a *cis* addition. Here the attack of both carbons is facilitated by the ease of approach from the α side, and as a consequence, the reaction is accomplished easily and completely with the formation of but a single product, the 11α,12α-epoxide.

Many other instances of the α attack could be cited. All of the reactions at C-12 in the $\Delta^{9,11}$ cholenic acids as well as the 3,9-epoxy derivatives conform to the generalization and of these compounds Mattox, *et al.*,[28] state: "It has not been possible to accomplish a conversion of any compound with the 12α configuration into one with a 12β arrangement by a replacement reaction. Regardless of the configuration at C-12 of the starting material, the entering substituent becomes attached to the α side of the molecule."

These examples then serve as an illustration of the general validity of the rule. It should find its greatest utility in the prediction of the stereochemical course of a reaction especially in the synthesis of adrenal cortical hormones. Moreover, the choice of proper experimental conditions for a reaction may often be governed by configuration. Certain extensions of the principle have already been encountered in this laboratory and will be described in detail in later communications.

We wish to express our appreciation to Dr. Willard Hoehn, University of Kansas, Kansas City, Missouri, for a gift of equilibrated pregnanolone.

Summary

1. Oxidation of 3α-acetoxy-17β-pregnan-20-one with perbenzoic acid yields 3α,17β-etiocholanediol diacetate; under the same circumstances 3α-acetoxy-17α-pregnan-20-one is converted to 3α,17α-etiocholanediol diacetate. The reaction therefore takes place without inversion at C-17.

2. The procedure is useful for the determination of configuration as well as for preparative purposes.

3. The configuration of C-12 and C-17 of the steroid nucleus is discussed and attention is drawn to certain regularities in reactions at these positions, which permit the prediction of the stereochemical course of many reactions.

4. The configuration of C-17 alcohols such as testosterone, estradiol and the related products can now be assigned with certainty; these natural products are 17β compounds.

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(23) Turner, Mattox, Engel, McKenzie and Kendall, *J. Biol. Chem.*, **166**, 345 (1946).

(24) Engel, Mattox, McKenzie, McGuckin and Kendall, *ibid.*, **162**, 565 (1946).

(25) Ott and Reichstein, *Helv. Chim. Acta*, **26**, 1799 (1943).

(26) Press and Reichstein, *ibid.*, **25**, 878 (1942).

(27) McKenzie, McGuckin and Kendall, *J. Biol. Chem.*, **162**, 555 (1946).

(28) Mattox, Turner, McKenzie, Engel and Kendall, *ibid.*, **173**, 283 (1948).