[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY, UNIVERSITY OF WASHINGTON]

Preparation of Ethylenedioxy Derivatives of Ketosteroids by Exchange Dioxolanation. An Improved Synthesis of Testosterone from Δ^4-Androstene-3,17-dione[1]

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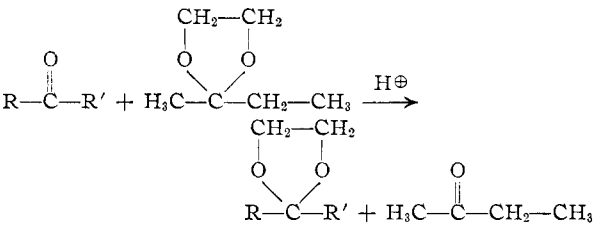
Steroidal ketones have been shown to undergo acid-catalyzed exchange with 2-methyl-2-ethyl-1,3-dioxolane or 2,2-dimethyl-1,3-dioxolane (cyclic ethyleneketals of butanone or acetone) to form ethylenedioxy derivatives in yields equal or superior to those obtained by the direct method. Exchange dioxolanation appears to be more subject to steric and electronic effects than direct dioxolanation, the reactivity order found being: saturated 3- and 20-ketones > α,β-unsaturated 3- and 20-ketones > 2- or 4-monobromo-3-ketones >> (no reaction) 17-ketones, 2,4-dibromo-3-ketone. Inertness of the 17-keto group has enabled selective conversion of Δ^4-androstene-3,17-dione into its 3-ethylenedioxy derivative which furnished testosterone on reduction of the unblocked 17-keto group and subsequent hydrolysis.

Blocking of ketone groups by formation of ethylenedioxy derivatives (cyclic ethylene glycol ketals; 1,3-dioxolanes) has proved to be a valuable adjunct to synthesis[4] and during recent years has become increasingly important in the steroid series.[5] Ethylenedioxy derivatives are usually prepared[4-6] by refluxing the ketone and excess ethylene glycol in benzene, toluene or ethylene dichloride solvent in the presence of acid catalysts (*p*-toluenesulfonic or sulfuric acid) and the water by-product continuously removed by slow distillation or collection of the condensate in a Dean–Stark phase separator. It has now been found[7] that steroidal ketones may be converted into their ethylenedioxy derivatives by a new method, exchange dioxolanation, which involves acid-catalyzed transfer of the ethylene glycol portion of simple 2,2-dialkyl-1,3-dioxolanes, such as 2,2-dimethyl-1,3-dioxolane (acetone ethyleneketal) or, better, 2-methyl-2-ethyl-1,3-dioxolane (butanone ethyleneketal), with the ketones either in an inert solvent,



as benzene, or simply in excess reagent. Conduct of the reaction and isolation of the product are simple, the reagent dioxolanes are prepared readily by direct dioxolanation, and yields of 70–96% have been realized. The generality of the exchange dioxolanation reaction has been demonstrated by its application to a considerable variety of steroidal ketones (Table I).

Exchange dioxolanation has furnished the ethylenedioxy derivatives of ketosteroids in yields comparable or, in most cases, superior to those obtained by the usual, direct dioxolanation method. Conversions realized by the exchange dioxolanation method are: saturated 3-ketones (cholestanone, coprostanone), 96%; Δ^4-3-ketones (testosterone, testosterone propionate, Δ^4-androstene-3,17-dione, progesterone), 70–78%; 20-ketone (progesterone), 71%; Δ^{16}-20-ketone ($\Delta^{5,16}$-pregnadien-3β-ol-20-one), 76%; 17-ketones (estrone, androstan-3α-ol-17-one acetate, Δ^4-androstene-3,17-dione dehydroepiandrosterone), no reaction[8]; 2-bromo-3-ketone (2-bromocholestanone), 78%; 4-bromo-3-ketone (4-bromoetiocholan-17β-ol-3-one acetate), 58%; 2,4-dibromo-3-ketone (2,4-dibromocholestanone), no reaction.[9]

(1) Presented before the Division of Organic Chemistry at the Fourth Northwest Regional Meeting of the American Chemical Society, Seattle, Wash., June 8, 1951 (*cf. Chem. Eng. News*, **89**, 2747 (1951)).

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(4) (a) E. Salmi, *Ber.*, **71**, 1803 (1938); (b) E. Salmi and V. Rannikko, *ibid.*, **72**, 600 (1939); (c) M. Kühn, *J. prakt. Chem.*, [2] **156**, 103 (1940); (d) A. Rossi and A. Lauchenauer, *Helv. Chim. Acta*, **30**, 1501 (1947); (e) M. Stoll, J. Hulstkamp and A. Rouvé, *ibid.*, **31**, 543 (1948); (f) M. Sulzbacher, E. Bergmann and E. R. Pariser, THIS JOURNAL, **70**, 2827 (1948); (g) F. Fleck, A. Rossi and H. Schinz, *Helv. Chim. Acta*, **32**, 998 (1949); (h) A. Lauchenauer and H. Schinz, *ibid.*, **32**, 1265 (1949); (i) L. Williman and H. Schinz, *ibid.*, **32**, 2151 (1949); (j) E. Vogel and H. Schinz, *ibid.*, **33**, 120 (1950); (k) M. W. Cronyn and J. E. Goodrich, THIS JOURNAL, **74**, 3331 (1952).

(5) (a) E. Fernholz and H. E. Stavely, Abstracts, 102nd Meeting of Am. Chem. Soc., Atlantic City, N. J., 39M (1941); (b) H. Köster and H. Inhoffen, U. S. Patent 2,302,636 (Nov. 17, 1942); (c) E. Fernholz, U. S. Patents 2,356,154 (Aug. 22, 1944) and 2,378,918 (June 26, 1945); (d) P. L. Julian, E. W. Meyer and I. Ryden, THIS JOURNAL, **72**, 367 (1950); (e) J. W. Ralls, W. C. Wildman, K. E. McCaleb and A. W. Wilds, Abstracts, 119th Meeting of Am. Chem. Soc., Cleveland, Ohio, 100M (1951); (f) A. W. Wilds, Abstracts, 120th Meeting of Am. Chem. Soc., New York, N. Y., 20M (1951); (g) R. Antonucci, S. Bernstein, R. Littell, K. J. Sax and J. H. Williams, *J. Org. Chem.*, **17**, 1341 (1952); (h) R. Antonucci, S. Bernstein, R. Lenhard, K. J. Sax and J. H. Williams, *ibid.*, **17**, 1369 (1952); (i) R. Antonucci, S. Bernstein, M. Heller, R. Lenhard, R. Littell and J. H. Williams, *ibid.*, **18**, 70 (1953); (j) G. Rosenkranz, J. Pataki and C. Djerassi, *ibid.*, **17**, 290 (1952); (k) E. Oliveto, T. Clayton and E. B. Hershberg, THIS JOURNAL, **75**, 486 (1953); (l) G. I. Poos, G. E. Arth, R. E. Beyler and L. H. Sarett, *ibid.*, **75**, 422 (1953); (m) R. M. Lukes, G. I. Poos, R. E. Beyler, W. F. Johns and L. H. Sarett, *ibid.*, **75**, 1707 (1953); (n) J. M. Constantin, A. C. Haven, Jr. and L. H. Sarett, *ibid.*, **75**, 1716 (1953); (o) L. H. Sarett, W. F. Johns, R. E. Beyler, R. M. Lukes, G. I. Poos and G. E. Arth, *ibid.*, **75**, 2112 (1953); (p) H. L. Herzog, M. A. Jevnik, M. E. Tully and E. B. Hershberg, *ibid.*, **75**, 4425 (1953).

(6) It has also been reported (ref. 5b) that ethylenedioxy derivatives may be prepared by the Lewis acid-catalyzed reaction of ethylene oxide with ketones.

(7) Constantin, Haven and Sarett (ref. 5n) have recently reported the application of the present method, using the dioxolane of mesityl oxide, for the preparation of the ethylenedioxy derivatives of 11-ketoprogesterone, dehydrocorticosterone acetate and cortisone acetate.

(8) 17-Ketones were inert only when pure anhydrous 2-methyl-2-ethyl-1,3-dioxolane, carefully purified by distillation through an efficient column, was employed. Reagent containing 0.5% ethylene glycol was found to react readily with 17-ketosteroids.

(9) Direct dioxolanation has given the following conversions: saturated 3- and/or 20-ketones (refs. 5c,k), 85–100%; Δ^4-3-ketones (refs. 5g,h,i,k,m), 32–47% and in one case 67%; Δ^{16}-20-ketone (ref. 5d), 51%; saturated 6-ketone (ref. 5c), 85%; saturated 17-ketone (refs. 5b,c), 15%; saturated 11-ketone (refs. 5i,k,n), no reaction. While these reported yields are probably not maximal in all cases, the generally better results obtained by the exchange dioxolanation method are probably significant. Since both methods employ equilibrium-shifting conditions, yield differences may originate from unequal form-

TABLE I

ETHYLENEDIOXY DERIVATIVES OF KETOSTEROIDS PREPARED BY EXCHANGE DIOXOLANATION

	Ketosteroid reactant	Position of ethylenedioxy formation[a]	Reacn. method[b]	Reacn. time, hr.	Yield, %	M.p., °C.	$[\alpha]^{24\pm1}D$ ($CHCl_3$)	Product formula	Carbon, % Calcd.	Carbon, % Found	Hydrogen, % Calcd.	Hydrogen, % Found
1	Coprostanone	3	A, C,	4, 8	96, 96	51–52	+27.6°	$C_{29}H_{50}O_2$	80.9	80.9	11.7	11.9
2	Cholestanone	3	A, C	4, 8	96, 96	113[c]	+21.6	$C_{29}H_{50}O_2$	80.9	80.7	11.7	11.6
3	Δ^4-Cholestenone	3	B	7	84	131–132[d]	−22.8[d]	$C_{29}H_{48}O_2$	81.3	80.9	11.3	11.0
4	Testosterone	3	B	6	78	183–184[e]	−43.1	$C_{21}H_{32}O_2$	75.8	75.5	9.7	9.8
5	Testosterone propionate	3	B	7	70	201–202	−46.5	$C_{24}H_{36}O_4$	74.2	74.3	9.3	9.2
6	Δ^4-Androstene-3,17-dione	3	B	5.5	74	197–198[e]	+15.4	$C_{21}H_{30}O_2$	76.3	76.6	9.2	9.2
7	Progesterone	3, 20	B, C, E	5, 10, 7	71, 68, 71	180–181[e]	−27.0[e]	$C_{25}H_{38}O_4$	74.7	74.3	9.5	9.4
8	$\Delta^{5,16}$-Pregnadien-3β-ol-20-one 3-acetate	20	B	20	76	157–158	−71.1	$C_{24}H_{36}O_4$	75.0	75.2	9.1	9.1
9	16,17-Oxido-Δ^5-pregnen-3β-ol-20-one 3-acetate	20	D	144	75	191–193[e]	−37.1[e]	$C_{24}H_{38}O_5$	72.0	72.2	8.7	8.9
10	Estrone	No reacn.	D	144	No reacn.							
11	Androstan-3α-ol-17-one 3-acetate	No reacn.	D	144	No reacn.							
12	Dehydroepiandrosterone	No reacn.	D	144	No reacn.							
13	2-Bromocholestanone	3	D	48	78	128–129	+23.4	$C_{29}H_{49}O_2Br$	68.3	68.3	9.7	9.8
14	4-Bromoetiocholan-17β-ol-3-one 17-acetate	3	D	72	58	204–205	−23.3	$C_{23}H_{35}O_4Br$	60.8	61.5	7.7	8.3
15	2,4-Dibromocholestanone	No reacn.	D	144	No reacn.							
16	Etiocholan-17β-ol-3-one 17-acetate	3	F	18	81	120		$C_{23}H_{38}O_4$	73.4	73.2	9.7	9.8

[a] 17-Ketones and a 2,4-dibromo-3-ketone failed to react. [b] Methods A, B, C and D employ 2-methyl-2-ethyl-1,3-dioxolane, method E uses 2,2-dimethyl-1,3-dioxolane, and method F utilizes 2-methyl-1,3-dioxolane; see Experimental for details. [c] Reported (ref. 5c), m.p. 115°. [d] Reported (refs. 5b,c,g,l), m.p. 132°, 133°, 134–135°; $[\alpha]D$, −28°, −31.4° at 30°, −25.5° at 25°. [e] Previously reported physical constants given in Experimental.

Various reaction periods, and even modified reaction methods (*vide infra*), were required for maximum conversion of different types of steroidal ketones into their ethylenedioxy derivatives by the exchange dioxolanation procedure, and reaction times were found to be markedly dependent on steric factors and, to a lesser extent, on electronic effects. Relative reactivity orders (and reaction times for maximum conversion by simplest effective reaction method, *cf.* Table I) were: unhindered, saturated 3- or 20-ketones (4–5 hr.) > Δ^4-3-ketones (5–7 hr.) > Δ^{16}-20-ketone (20 hr.) > 2- or 4-monobromo-3-ketone (48–72 hr.) > hindered, saturated 20-ketone (16,17-oxido-Δ^5-pregnen-3β-ol-20-one acetate) (144 hr.) >> 17-ketones[8] or 2,4-dibromo-3-ketone (no reaction in 144 hr.). Exchange dioxolanation, apparently due to the greater bulk of the dioxolane reactants, has proved to be somewhat more sensitive to structural (steric and electronic) factors of the ketone than direct dioxolanation[10] and, consequently, more adaptable to selective blocking of polyketonic steroids and similar compounds.

Ease with which exchange dioxolanation occurred determined the preparative procedure to be followed. Reaction of unhindered, saturated 3- and 20-ketones could be accomplished by simple boiling with the dioxolane reactant in the presence of *p*-toluenesulfonic acid catalyst. With α,β-unsaturated 3-ketones, slow distillation to accomplish removal of the by-product ketone, butanone or acetone, was desirable but not necessary. Less reactive, sterically-hindered ketones undergo the exchange reaction satisfactorily only by the more effective, equilibrium-shifting method of butanone or acetone removal by a stripping technique.

In attempts to accelerate reaction on sterically-hindered and deactivated carbonyl groups various catalysts other than *p*-toluenesulfonic acid and other, less bulky, dioxolanes have been examined. Mesitylenesulfonic acid and anhydrous zinc chloride proved unsatisfactory in the reaction of cholestenone with 2-methyl-2-ethyl-1,3-dioxolane; some butanone was liberated but no crystalline ethylenedioxy product could be isolated. *p*-Nitrobenzenesulfonic acid and picrylsulfonic acid were tried only in the reaction of 16,17-oxido-Δ^5-pregnen-3β-ol-20-one acetate with the methylethyldioxolane, and decomposition, presumably due to reaction at the epoxide ring, was observed. Attempted use of the less highly substituted reactant, 1,3-dioxolane (formaldehyde ethyleneketal) in an exchange reaction with cholestanone gave only polymeric products.[11] 2-Methyl-1,3-dioxolane (acetaldehyde ethyleneketal), by simple boiling with a saturated ketone, etiocholan-17β-ol-3-one acetate, in the presence of *p*-toluenesulfonic acid catalyst gave the ethylenedioxy derivative in 81% yield, but with α,β-unsaturated ketones no crystalline products could be obtained. 2,2-Dimethyl-1,3-dioxolane (acetone ethyleneketal) gave essentially the same results as 2-methyl-2-ethyl-1,3-dioxolane (butanone ethyleneketal) but the higher boiling point of the latter led to somewhat faster reaction.

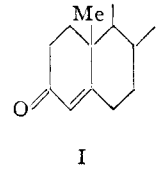
Evidence has been presented, originally by Fernholz and Stavely[5a,c] and more recently by Williams,

ation of by-products. It is reasonable, and some evidence has been obtained (ref. 4k), that enol ether formation (mono or bis) may be an important competing reaction, particularly with the facile enolizing Δ^4-3-ketones. The observation of Fernholz (ref. 5c) that Δ^4-androstene-3,17-dione may be recovered quantitatively by hydrolysis of the oily by-products resulting from its direct dioxolanation is in accord with this contention.

(10) Direct dioxolanation has been found to occur with approximately equal ease with saturated or α,β-unsaturated 3-, 6- and 20-ketosteroids (refs. 5a,b,c,g,h,k); 17-ketones react less readily (refs. 5a,b,c) and 11-ketones or hindered 20-ketones have failed to react (refs. 5h,i,n). The most striking differences in reactivity in the two dioxolanation methods are 17-ketones, which react fairly rapidly in the direct method but are completely unreactive in the exchange method (*cf.*, ref. 8), and a hindered 20-ketone, 16,17-oxido-Δ^5-pregnen-3β-ol-20-one acetate, which formed 51% of the ethylenedioxy derivative in 4 hours by the direct method (ref. 5d) but required 144 hours for 76% conversion by the exchange method.

(11) W. F. Gresham, U. S. Patent 2,395,265 (Feb. 19, 1946), has reported that 1,3-dioxolane undergoes polymerization in the presence of acid catalysts.

et al.,[5g,h] and by Sarett, *et al.*,[5l,n] that Δ^4-3-ketones (I) undergo double bond migration on direct dioxolanation to give Δ^5-3-ethylenedioxy products (β,γ-unsaturated ethyleneketals) (II). In the present studies, confirmation of the recent conclusion of



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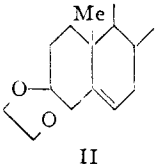

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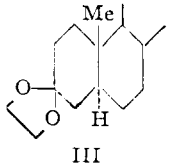

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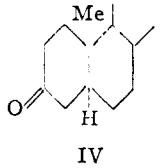


IV

Constantin, Haven and Sarett[5n] that similar double bond migration results also on exchange dioxolanation has been obtained by the observation that 3-ethylenedioxy derivatives of several Δ^4-3-ketones (cholestenone, testosterone, progesterone, Δ^4-androstene-3,17-dione) prepared by the exchange method all possess physical constants in agreement with those formed by the direct method. No attempt has been made in the present work to establish the structure of the dioxolane product from another type of α,β-unsaturated steroidal ketone, $\Delta^{5,16}$-pregnadien-3β-ol-20-one acetate.[12]

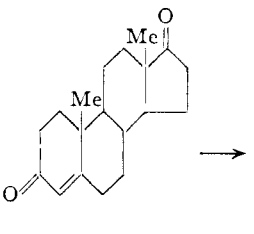
Ethylenedioxy derivatives have proven to be versatile blocking groups[13] and have been employed in the present studies to perform certain steroidal transformations. The isomerization observed in the dioxolanation of Δ^4-3-ketones may be utilized to advantage for their conversion into saturated 3-ketones with *allo* configuration; in confirmation of the brief statement (without experimental details) of Fernholz,[5c] Δ^4-3-cholestenone on exchange dioxolanation and palladium catalyst hydrogenation has been found to furnish exclusively 3-ethylenedioxycholestane[14] (I → II → III → IV).

(12) Generalization about the occurrence of isomerization in dioxolanation of α,β-unsaturated ketones is not yet justified; only the very labile Δ^4- and $\Delta^{4,7}$-3-ketones in the steroid and related series and, to a lesser extent, the moderately labile mesityl oxide have shown this migration (refs. 5g,h,i,l,n).

(13) Dioxolanes have been found to be stable in many different chemical operations: (1) reduction by catalytic hydrogen (refs. 4d,e,k,5c,g,h), by sodium and alcohol (refs. 4i,5g,h), by lithium aluminum hydride (refs. 5d,i,l), and by sodium borohydride (ref. 5k); (2) oxidation by Oppenauer method (refs. 5d,g,l), by chromic anhydride–pyridine complex (refs. 5l,m,n,o), by silver oxide (ref. 4e), and by osmium tetroxide (ref. 5o); (3) esterification (refs. 5g,i,l); (4) saponification (refs. 5d,g,l); (5) bromine addition (ref. 4e); (6) allylic bromination (refs. 5g,h), dehydrobromination (refs. 4e,5g,h); (7) cyanhydrin formation (ref. 4k); (8) Grignard reactions (ref. 4i; *cf.* 4i and J. F. Arens and D. A. VanDorp, *Rec. trav. chim.*, **65**, 729 (1946), for attempted preparations of Grignard reagents from α- and β-halo ethylenedioxy compounds); (9) Reformatsky reactions (ref. 4k); (10) base-catalyzed active methylene condensations (refs. 4i,e,5m,o). Ethylenedioxy groups are usually stable under basic conditions (but see ref. 4k) and converted to ketones by acid hydrolysis (ref. 4,5; weak acids may allow selective hydrolysis, ref. 5i) or by acid-catalyzed exchange with acetone (ref. 4i; H. Schinz and G. Schappi, *Helv. Chim. Acta*, **30**, 1265 (1947); C. A. Grob, W. Jundt and H. Wicki, *ibid.*, **32**, 2247 (1947)).

(14) This observation affords additional support for the formulation of the dioxolane of Δ^4-3-cholestenone as 3-ethylenedioxy-Δ^5-cholestene.

Exchange dioxolanation also provides a simple, convenient synthesis of testosterone from the readily available Δ^4-androstene-3,17-dione which



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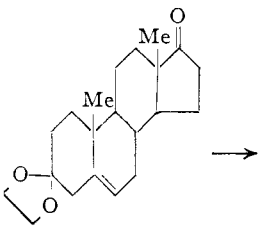

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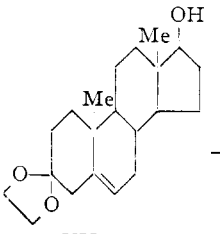

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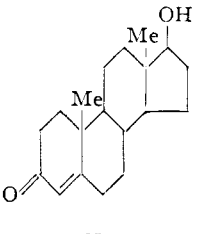

VIII

compares favorably with the analogous method employing 3-enol ether blocking[15] or the recently reported sequence involving the 2,3-dihydropyran derivative of dehydroepiandrosterone.[16] Conversion of Δ^4-androstene-3,17-dione to testosterone by means of 3-ethylenedioxy blocking was accomplished first by Fernholz and Stavely,[5a,c] but the present method, utilizing exchange dioxolanation for the selective preparation of the necessary 3-monoethylenedioxy derivative, overcomes the difficulties encountered in their direct dioxolanation step and makes this conversion sequence efficient and practical.[17]

Δ^4-Androstene-3,17-dione (V) on exchange dioxolanation was converted in 74% yield[18] into 3-ethylenedioxy-Δ^5-androsten-17-one[8] (VI) which was reduced at C-17 by hydrogen with Raney nickel catalyst in ethanol solution or by lithium aluminum hydride in tetrahydrofuran solution to 3-ethylenedioxy-Δ^5-androsten-17β-ol (VII, testosterone 3-dioxolane) in 90% yield. Acid-catalyzed exchange with acetone[13] gave testosterone (VIII), identical with authentic material, in quantitative yield or in 67% over-all yield from V. The reactions are few and specific and are performed conveniently in high yield. To further establish

(15) A. Serini and H. Köster, *Ber.*, **71**, 1766 (1938); G. Rosenkranz, St. Kaufmann and J. Romo, THIS JOURNAL, **71**, 3689 (1949).

(16) A. C. Ott, M. F. Murray and R. L. Pedersen, *ibid.*, **74**, 1239 (1952).

(17) Fernholz and Stavely obtained only *ca.* 25% over-all yield of testosterone from androstenedione; their direct dioxolanation furnished only a low yield of mixed dioxolane products from which *ca.* 25% of the desired 3-monodioxolane and *ca.* 15% of the 3,17-bis-dioxolane could be separated by chromatographic adsorption. Herzog, Jevnik, Tully and Hershberg (ref. 5p) on recent very careful examination of the direct dioxolanation of androstenedione with only one equivalent of ethylene glycol have found that both 3- and 17-monodioxolane and 3,17-bis-dioxolane products are formed, the relative amounts being dependent on the steroid–acid catalyst ratio; under most favorable conditions only 50% of the 3-monodioxolane product was isolated, chromatographic adsorption again being utilized for separation.

(18) Acid hydrolysis of the mother liquors, containing presumably enol ethers and other by-products (*cf.* ref. 9), was not performed but would provide considerable recoverable starting material and consequent higher conversions.

the structure of the intermediate, it has been shown that propionylation of the reduction product with propionic anhydride in excess pyridine produced 3-ethylenedioxy-Δ^5-androsten-17β-ol 17-propionate, identical with dioxolanation product of testosterone propionate.

Experimental[19]

Preparation of 1,3-Dioxolanes. **2-Methyl-2-ethyl-1,3-dioxolane.**—A mixture of butanone (72.1 g., 89.6 ml., 1.00 mole; dried over Drierite and decanted), ethylene glycol (62.1 g., 55.8 ml., 1.00 mole), *p*-toluenesulfonic acid monohydrate (0.5 g.) and benzene (50 ml.) was heated under reflux in a modified Dean–Stark phase separator[20] until no more aqueous phase separated (*ca.* 1.1 moles, 20 ml., in about 30 hours). After neutralization by addition of excess anhydrous sodium carbonate, the unfiltered reaction mixture was subjected to fractional distillation in a packed column (38 × 1 cm. glass helices) with a total condensation, partial take-off head. After removal of the benzene, an intermediate fraction of slightly impure product (12 g., $n^{25.0}$D 1.4162) and a main fraction of 2-methyl-2-ethyl-1,3-dioxolane (90 g., 78%, b.p. 116.5–117°, $n^{24.8}$D (1.4087) were collected.[21] Physical constants previously reported are: b.p. 118–118.5°, n^{20}D 1.4110[22]; b.p. 115.4–116.2° (763 mm.), n^{20}D 1.4096.[4b]

2,2-Dimethyl-1,3-dioxolane.—Acetone (58 g., 1.00 mole), ethylene glycol (62.1 g., 1.00 mole), *p*-toluenesulfonic acid monohydrate (0.5 g.), and benzene (150 ml.) were allowed to react as described above. Redistillation of the main fraction through a glass helices packed column gave pure 2,2-dimethyl-1,3-dioxolane (66 g., 65%, b.p. 91.5–93°, n^{20}D 1.3995). *Physical constants previously reported are:* b.p. 92–92.5°, n^{20}D 1.4000.[22]

2-Methyl-1,3-dioxolane.—A mixture of acetaldehyde (88 g., 114 ml., 2.00 moles), ethylene glycol (124 g., 111 ml., 2.00 moles) and *p*-toluenesulfonic acid monohydrate (2.0 g.) was heated overnight under reflux on a steam-bath. After neutralization with solid potassium carbonate, the methyldioxolane–water azeotrope, b.p. 78°, was collected on distillation through the packed (glass helices) column. The azeotrope was shaken with anhydrous potassium carbonate, the upper layer separated and allowed to stand with additional potassium carbonate overnight. Distillation furnished 2-methyl-1,3-dioxolane (80 g., 45%, b.p. 82°, $n^{25.2}$D 1.4072; reported[23] b.p. 82–83°, n^{20}D 1.3970).

1,3-Dioxolane.—To a mixture of paraformaldehyde (60 g.) and ethylene glycol (150 g.) was added concentrated hydrochloric acid (4 ml.) and the resultant mixture heated under reflux on a steam-bath for 16 hours. When it was found that a sample did not separate into two layers on shaking with a few pellets of potassium hydroxide, *p*-toluenesulfonic acid (1 g.) catalyst was added and the mixture boiled for an additional 24 hours. Fractional distillation furnished the dioxolane–water azeotrope (125 g., *ca.* 84%, b.p. 71°) which was shaken with solid potassium carbonate, the upper layer separated and shaken once more with potassium carbonate before distillation to yield the anhydrous product (85 g., 57%, b.p. 76°, $n^{25.3}$D 1.4010; reported[24] b.p. 76°, $n^{20}\alpha$ 1.3974, $n^{20}\gamma$ 1.4073).

General Procedures for Exchange Dioxolanations. (A) **By Refluxing with Undiluted 2-Methyl-2-ethyl-1,3-dioxolane**[25] (suitable for saturated 3- and 20-ketones). **3-Ethylenedioxycoprostane.**—A solution of coprostanone (1.0 g.) and *p*-toluenesulfonic acid monohydrate (15 mg.) in 2-methyl-2-ethyl-1,3-dioxolane (20 ml.) was boiled under reflux and anhydrous conditions for 4 hours. The cooled reaction mixture was diluted with benzene, washed successively with 5% aqueous sodium bicarbonate and with water, dried over sodium sulfate and concentrated to dryness under reduced pressure. Crystallization of the residue from methanol containing a drop of pyridine gave 3-ethylenedioxycoprostanone (1.06 g., 96%, m.p. 51–52°, $[\alpha]^{24}$D +27.6°).

(B) **By Distillation with Undiluted 2-Methyl-2-ethyl-1,3-dioxolane**[25] (best method for α,β-unsaturated 3- and 20-ketones[26]). **3,20-Bis-ethylenedioxy-Δ^5-pregnene.**—A mixture of progesterone (1.0 g.), *p*-toluenesulfonic acid monohydrate (30 mg.; larger amounts of catalyst are needed for α,β-unsaturated ketones) and 2-methyl-2-ethyl-1,3-dioxolane (20 ml.) was heated and the liberated butanone, admixed with the reactant dioxolane, distilled slowly through a small Claisen–Vigreux column at atmospheric pressure for a period of 5 hours (8 ml. distillate collected). Work-up of the reaction mixture as described in A gave the bis-dioxolane of progesterone, 3,20-bis-ethylenedioxy-Δ^5-pregnene (0.91 g., 71%, m.p. 180–181°, $[\alpha]^{24}$D −27.0°; reported[5h] m.p. 181–182°, $[\alpha]^{31}$D −28.9°).

(C) **By Distillation with Diluted 2-Methyl-2-ethyl-1,3-dioxolane**[25] (suitable for saturated or α,β-unsaturated ketones). **3,20-Bis-ethylenedioxy-Δ^5-pregnene.**—A solution of progesterone (1.0 g.), *p*-toluenesulfonic acid monohydrate (30 mg.), 2-methyl-2-ethyl-1,3-dioxolane (20 ml.) and benzene[27] (20 ml.) was distilled slowly as in B (15 ml. collected). Work-up of the reaction mixture as in A gave the bis-dioxolane of progesterone (0.88 g., 68%, m.p. 180–181°).

(D) **By Stripping Distillation with Undiluted 2-Methyl-2-ethyl-1,3-dioxolane** (best for sterically-hindered ketones). **20-Ethylenedioxy-16,17-oxido-Δ^5-pregnen-3β-ol 3-Acetate.**—A solution of 16,17-oxido-Δ^5-pregnen-3β-ol-20-one acetate[5d] (510 mg.) in 2-methyl-2-ethyl-1,3-dioxolane (15 ml.) containing *p*-toluenesulfonic acid monohydrate (7 mg.) was heated in a flask fitted with a twisted wire gauze distillation column[28] and a partial take-off head. A few drops of distillate was collected at initiation of the reaction to remove any moisture and the take-off stopcock closed. Progress of the reaction was indicated by temperature drop at the top of the column and it was necessary to remove only a few drops twice daily. Simultaneously with collection of each distillate a crystal of *p*-toluenesulfonic acid was added at the top of the column (total additional catalyst, 7 mg.). After the column had been stripped in this manner for 6 days, no further temperature drop was noted. Work-up of the reaction as in A and crystallization from methanol (1 drop pyridine) gave the dioxolane product (430 mg., 76%, m.p. 185°). Two recrystallizations from benzene–methanol gave pure 20-ethylenedioxy-16,17-oxido-Δ^5-pregnen-3β-ol 3-acetate (m.p. 191–193°, $[\alpha]^{24}$D −37.1°; reported[5d] m.p. 195–197°, $[\alpha]^{27}$D −37.8°).

(E) **With 2,2-Dimethyl-1,3-dioxolane.**[25] **3,20-Bis-ethylenedioxy-Δ^5-pregnene.**—Slow distillation of a solution of progesterone (1.0 g.) and *p*-toluenesulfonic acid monohydrate (20 mg.) in 2,2-dimethyl-1,3-dioxolane (15 ml.) as in B for 7 hours (5 ml. collected), work-up as in A, and crystallization of the crude product from methanol (1 drop pyridine) furnished the bis-dioxolane of progesterone (0.91 g., 71%, m.p. 180–181°).

(F) **With 2-Methyl-1,3-dioxolane.**[25] **3-Ethylenedioxyetiocholan-17β-ol 17-Acetate.**—A mixture of etiocholan-17β-ol-3-one acetate (1.0 g.), *p*-toluenesulfonic acid mono-

(19) Boiling points are uncorrected. Melting points were determined on a Fisher block and are uncorrected. Optical rotations were determined in chloroform using 1% solutions in 1-dm. semi-micro tubes.

(20) The usual phase separator (E. W. Dean and D. D. Stark, *Ind. Eng. Chem.*, **12**, 486 (1920)) proved ineffective in direct dioxolanations. Best results were obtained by modification of the apparatus by inclusion of a small condenser to cool the condenate before entrance into the collector (*cf.* ref. 4k) and by introduction of small long-stemmed funnel in the collector to circulate the cooled condensate through the contents (S. Natelson and S. Gottfried, *Org. Syntheses*, **23**, 38 (1943)), for continuous re-extraction of codistilled ethylene glycol and ketone.

(21) Complete purification may be attained alternately by redistillation from lithium aluminum hydride of material once distilled through an ordinary Claisen distillation apparatus.

(22) A. A. Petrov, *J. Gen. Chem. U.S.S.R.*, **10**, 981 (1940); *C. A.*, **25**, 3603 (1941).

(23) W. J. Croxall, F. J. Glavis and H. T. Neher, THIS JOURNAL, **70**, 2805 (1948).

(24) H. T. Clarke, *J. Chem. Soc.*, **101**, 1804 (1912).

(25) 2,2-Dimethyl-1,3-dioxolane may be substituted for 2-methyl-2-ethyl-1,3-dioxolane in procedures A, B, or C (see E for an example) but the higher boiling point of the latter permits easier separation from benzene during preparation and more favorable reaction temperature. 2-Methyl-1,3-dioxolane has proved satisfactory for saturated keto-steroids but unsatisfactory for α,β-unsaturated ketones (see F for an example).

(26) For Δ^{16}-20-ketones a reaction time of approximately 20 hours is necessary.

(27) Methylcyclohexane or toluene may be substituted for benzene and the reaction time reduced to 8 hours.

(28) J. M. Bower, Jr., and L. M. Cooke, *Ind. Eng. Chem., Anal. Ed.*, **15**, 291 (1943).

hydrate (10 mg.) and 2-methyl-1,3-dioxolane (10 ml.), which was boiled as in A for 18 hours and worked up as in A, gave from methanol (1 drop of pyridine) 3-ethylenedioxyetiocholan-17β-ol acetate (0.92 g., 81%, m.p. 120°).

(G) **Attempted Dioxolanation with 1,3-Dioxolane.**—Cholestanone (1.0 g.) was dissolved in 1,3-dioxolane (10 ml.), *p*-toluenesulfonic acid monohydrate (10 mg.) added and the mixture boiled under reflux and anhydrous conditions for 5 hours. The usual work-up as in A gave only non-crystalline polymeric material[11] (3 g.).

Transformation of Ethylenedioxysteroids. Reduction of 3-Ethylenedioxy-Δ⁵-cholestene. 3-Ethylenedioxycholestane.—3-Ethylenedioxy-Δ⁵-cholestene (430 mg.) in absolute ethanol (20 ml.) was added to pre-hydrogenated 5% palladium–barium sulfate catalyst (40 mg.) suspended in absolute ethanol (15 ml.) and hydrogenated at atmospheric pressure. The theoretical amount of hydrogen (24.4 ml. at 24°, 760 mm.; 1.00 equiv.) was absorbed in 1.5 hours. The filtered solution on concentration under reduced pressure and cooling deposited 3-ethylenedioxycholestane (380 mg., 88%, m.p. 111–113°, $[\alpha]^{25}D$ +20.2°), identical with the product prepared directly from cholestanone.

Conversion of Δ⁴-Androstene-3,17-dione into Testosterone. 3-Ethylenedioxy-Δ⁵-androsten-17-one (VI).—A solution of Δ⁴-androstene-3,17-dione (1.0 g.) and *p*-toluenesulfonic acid monohydrate (15 mg.) in pure, carefully fractionated[8,21] 2-methyl-2-ethyl-1,3-dioxolane (16 ml.) was distilled slowly through a glass helices-packed column for 5.5 hours (10 ml. of distillate collected). Work-up as in A followed by crystallization from methanol (1 drop of pyridine) gave the 3-monodioxolane product, 3-ethylenedioxy-Δ⁵-androsten-17-one (840 mg., 74%, m.p. 197–198°, $[\alpha]^{24}D$ +15.4°; reported m.p. 199°[5b] and double m.p. of 194° and 202°[5c]). No attempt was made to recover androstenedione by acid hydrolysis of mother liquors.[18]

3-Ethylenedioxy-Δ⁵-androsten-17β-ol (VII).—3-Ethylenedioxy-Δ⁵-androsten-17-one (535 mg.) in absolute ethanol (40 ml.) was added to prehydrogenated W-4 Raney nickel catalyst[29] (0.5 g.) in absolute ethanol (25 ml.) and hydrogenation at atmospheric pressure ceased in 1 hour after the absorption of one equivalent (39.8 ml. at 27°, 760 mm.; 1.00 equiv.). Concentration of the filtered solution, after addition of 2-methyl-2-ethyl-1,3-dioxolane (5 ml.), under reduced pressure to remove the ethanol followed by refrigeration overnight yielded 3-ethylenedioxy-Δ⁵-androsten-17β-ol (472 mg., 90%, m.p. 181–182°).[30] The twice recrystallized (from methanol, 1 drop of pyridine) product (m.p. 183–184°, $[\alpha]^{24}D$ −41.7°) was identical with authentic testosterone dioxolane (m.p. and m.m.p. 182–183°, $[\alpha]^{24}D$ −43.1°) prepared by exchange dioxolanation.

Testosterone (VIII).—3-Ethylenedioxy-Δ⁵-androsten-17β-ol (1.0 g.) was dissolved in anhydrous acetone (50 ml.), *p*-toluenesulfonic acid monohydrate (50 mg.) added and the mixture boiled under reflux for 14 hours. Concentration of the resultant solution to a small volume (10 ml.) and precipitation with water gave a quantitative yield of slightly impure testosterone (0.87 g., 100%, m.p. 147–151°). Recrystallization from ether furnished the pure product (m.p. 152–154°, $[\alpha]^{24}D$ +109°), identical in all respects with authentic testosterone.

3-Ethylenedioxy-Δ⁵-androsten-17β-ol 17-Propionate.—A mixture of 3-ethylenedioxy-Δ⁵-androsten-17β-ol (500 mg.), pyridine (3.5 ml.) and propionic anhydride (1.5 ml.) was heated at 95° for 14 hours. Dimethylaniline (3 ml.) was added, the solution concentrated under reduced pressure almost to dryness and treated with methanol (5 ml.). Refrigeration overnight gave 3-ethylenedioxy-Δ⁵-androsten-17β-ol 17-propionate (505 mg., 86%, m.p. 196–198°); the once recrystallized (from methanol, 1 drop of pyridine) product (m.p. and m.m.p. 201–202°, $[\alpha]^{23}D$ −47.7°) was identical with a sample prepared by exchange dioxolanation of testosterone propionate.

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(29) A. Pavlic and H. Adkins, THIS JOURNAL, **68**, 1471 (1946).

(30) The same yield was obtained when the reduction was carried out by lithium aluminum hydride in tetrahydrofuran solution.

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[CONTRIBUTION FROM THE U. S. NAVAL MEDICAL RESEARCH INSTITUTE]

Nature of the Acetyl Cholinesterase Surface. I. Some Potent Competitive Inhibitors of the Enzyme[1a,b]

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Highly purified acetyl cholinesterase from electric eel tissue has been used in a test of the theory that one class of reversible, competitive inhibitors of the enzyme has as an essential feature of structure a locus of high electron density separated by roughly a $-CH_2CH_2-$ unit's distance from a polymethylated nitrogen atom (preferably quaternary). Specific compounds tested in this category include the N-(β-trimethylammonium)-ethyl derivatives of pyrrolidine and piperidine, as well as the corresponding dimethylamino compounds. These materials were found to inhibit competitively and to possess an anticholinesterase activity equal to or greater than that shown by eserine or prostigmine, as measured by the dissociation constants of the enzyme–inhibitor complexes. These data are discussed in terms of the corresponding fine structure of the enzyme surface. It has also been observed that the enzymatic hydrolysis rates for acetyl choline are virtually independent of ionic strength over the range 0.18–0.56, at a substrate concentration yielding the maximum rate, and that phosphate ions are not essential to enzymatic activity. Solution constituents which complex Mg(II) ion markedly reduce the activity.

Introduction

As part of a program designed to investigate the surface configuration of the enzyme acetyl cholinesterase (AChE), and ultimately to unravel details of the mechanism involved in its catalysis of the hydrolysis of acetylcholine (AC) in intact nervous tissue, the present study deals with the relative effects of some powerful inhibitors of the hydrolysis reaction. Working from the structures and the approximate distances of charge separation in these competitive inhibitors, and the assumption that these quantities are not perturbed on adsorption by the enzyme, certain tentative conclusions may be drawn as to the complementary fine structure of the enzymatic surface. These results constitute a logical extension of the excellent studies of Nachmansohn, Wilson and collaborators[2] leading to their postulation of the existence of two active sites per

(1) (a) The opinions in this paper are those of the authors, and do not necessarily reflect the views of the Navy Department. (b) Presented in part before the Division of Biological Chemistry, The American Chemical Society, Chicago, Illinois, September 6–11, 1953.

(2) For a detailed review of their work in this field, see D. Nachmansohn and I. B. Wilson, *Advances in Enzymol.*, **12**, 259 (1951).