A New Approach to the Characterization of Steroids Containing the 17 α -Ketol Side Chain

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(Received 29 July 1963)

When only minute quantities of a steroid are available for analysis, paper or thin-layer chromatography is frequently used to identify the compound and its derivatives. Most modifications of this approach require co-chromatography of a known steroid treated in a manner similar to the unknown. Occasionally, because a steroid is new or rare, a standard may not be available for comparison. Bush (1960, 1961) has pointed out that the chromatographic behaviour of steroids in a given solvent system changes in a consistent manner that is determined by alterations in the nature of the substituent groups. By using an analytical approach based on this generalization, Bush showed that it is possible tentatively to identify steroids without the use of standards. The theory and definitions that led to this method were first derived by Martin (1950) and Martin & Synge (1941), who showed that, as a first approximation, the change in partition coefficient resulting from the addition of functional groups to a molecule depends on the nature of the substituents and solvent pair, and is independent of the nature of the substituted molecule. A function of the partition coefficient expressed in terms of R_F has been called the R_M function by Bate-Smith & Westall (1950).

The change in R_M , i.e. ΔR_M , under specified conditions is a characteristic constant for a variety of substituents (Bate-Smith & Westall, 1950; Bradfield & Bate-Smith, 1950; Bremner & Kenten, 1951; Reichl, 1955; Bush & Hockaday, 1962). The ΔR_M approach was first intensively analysed as a technique for steroid characterization by Bush (1961), but appears to have been used for this purpose by few others (Brooks, Hunt, Long & Mooney, 1957; Kabasakalian & Basch, 1960). In the present paper, a systematic application of the ΔR_M concept to derivatives of corticosteroids is presented to illustrate a new approach to the characterization of the ketol side chain. It is shown that the R_M theory may be applied to thin-layer chromatography as well as to paper chromatography. The ΔR_M relationship is found to hold for two potentially interesting groups of corticosteroid derivatives, the 21-dehydro steroids and 17 β -carboxyl steroids (etienic acids).

MATERIALS AND METHODS

All steroids used were purchased from Steraloids Inc., New York, or contributed by Dr Abraham White. Although most of the steroids used were chromatographically homogeneous, some contained trace contaminants that did not interfere with the analytical procedures. All solvents and chemicals were of analytical reagent or spectrophotometric grade and were used without further purification. Silica gel, prepared according to the procedure of Stahl (1958), particle size 5–25 μ , and containing 13% of calcium sulphate, was purchased from E. Merck A.-G., Darmstadt, Germany. Thin-layer plates were prepared with a Camag apparatus (Arthur H. Thomas Co., New York). Wide-mouth screw-cap jars (25 cm. high $\times$ 15 cm. diam.) were purchased locally.

Terminology of steroids. The trivial names for steroids referred to are: cortisol, 11 β ,17 α ,21-trihydroxypregn-4-ene-3,20-dione; cortisone, 17 α ,21-dihydroxypregn-4-ene-3,11,20-trione; deoxycorticosterone, 21-hydroxypregn-4-ene-3,20-dione; 11-dehydrocorticosterone, 21-hydroxypregn-4-ene-3,11,20-trione; corticosterone, 11 β ,21-dihydroxypregn-4-ene-3,20-dione; 11-deoxycortisol, 17 α ,21-dihydroxypregn-4-ene-3,20-dione; Δ^1 -cortisone, 17 α ,21-dihydroxypregna-1,4-diene-3,11,20-trione; 9 α -fluorocortisol, 9 α -fluoro-11 β ,17 α ,21-trihydroxypregn-4-ene-3,20-dione; Triamcinolone, 9 α -fluoro-11 β ,16 α ,17 α ,21-tetrahydroxypregna-1,4-diene-3,20-dione; testosterone, 17 β -hydroxyandrost-4-en-3-one; 17 β -oestradiol, oestra-1,3,5(10)-triene-3,17 β -diol; oestrone, 3-hydroxyoestra-1,3,5(10)-trien-17-one; oestriol, oestra-1,3,5(10)-triene-3,16 α ,17 β -triol; progesterone, pregn-4-ene-3,20-dione; 21-dehydrocortisol, 11 β ,17 α -dihydroxy-3,20-dioxopregn-4-en-21-al; etienic acid corresponding to cortisol, 11 β ,17 α -dihydroxy-3-oxoandrost-4-ene-17 β -carboxylic acid. Other 21-dehydro steroids and etienic acids are named in a corresponding manner.

Preparation of steroid derivatives. (a) 21-Dehydro corticosteroids. Corticosteroid (1–4 mg.) was dissolved in 0.5 ml. of methanol; 0.5 ml. of 0.01M-cupric acetate in methanol was added, and the mixture was heated at 60° for 1 hr. then cooled to room temperature. Ethylenediaminetetraacetic acid (5 mg.) and 0.2 ml. of water were added and the suspension was shaken for 2 min. When heating was prolonged, small amounts of etienic acid were also formed. The derivatives were spotted directly on prepared silica-gel plates without further treatment.

(b) Etienic acids. Corticosteroid (1–4 mg.) was dissolved in 1 ml. of methanol; 0.05 ml. of 5N-sulphuric acid and 0.6 ml. of 0.03N-periodic acid were added and the mixture was heated at 60° for 1 hr. The resulting etienic acids were directly spotted on thin-layer plates.

(c) 16 α ,17 α -Acetonide. 9 α -Fluoro- Δ^1 -16 α -hydroxycortisol (Triamcinolone; E. R. Squibb and Sons, New Brunswick, N.J., U.S.A.) was dissolved in 2% hydrochloric acid in acetone. After 30 min. at 60°, acetone was removed under a stream of nitrogen. The acetonide was redissolved in methanol or ethanol, and treated as described under (a) or (b).

Preparation of thin-layer plates. Silica gel (30 g.) was stirred with 60 ml. of water for 3 min., then layered on glass plates (150 mm. $\times$ 50 mm.) at a thickness of 0.3 mm. Seven to nine plates were thus obtained. After 15 min. at room temperature, the plates were heated at 110° for 60 min., cooled in air for 30 min. and then spotted with the steroids 2.5 cm. from the base, by using a Camag plastic spotting guide (A. H. Thomas Co., New York). One to three 5 μ l. applications were usually sufficient. The plates were placed in wide-mouth screw-cap jars containing 200 ml. of solvent. The jars were closed and solvent was allowed to ascend for 100 mm.

Detection of spots. Air-dried plates were scanned with an ultraviolet lamp emitting radiation at 254 m μ . Steroids containing the Δ^4 -3-oxo function were detected as opaque spots against a green-fluorescent background. Steroids were also detected after spraying the plates with 0.5% guaiacolsulphonate in 50% sulphuric acid and heating at 110° until coloured spots appeared.

Solvent systems. These were as follows. System 1: ethyl acetate saturated with water at room temperature (23–26°). The jar containing solvent was equilibrated for 30 min. with 0.5 ml. of conc. hydrochloric acid contained in a beaker before adding the thin-layer plate. System 2: ethyl acetate–methylene chloride–aq. 88% formic acid (19:19:2, by vol.). System 3: chloroform–acetone–acetic acid (10:10:1, by vol.). System 4: chloroform–acetone–acetic acid (12:8:1, by vol.). System 5: chloroform–ethanol (25:1, v/v).

RESULTS

Effect of steroid concentration and the presence of other steroids on mobility. Preliminary experiments were performed to determine if chromatographic mobility were affected by changes in steroid concentration within the limits used in the present study. The distances moved by steroids on thin-layer plates were not affected by the concentration of steroid in the range 15–150 μ g. per spot. No streaking of spots at the higher concentrations of steroid was observed and all steroids moved as discrete well-defined areas, usually circular, with a tendency to become oval at R_F values above 0.85.

In the presence of impurities or other steroids, adsorption systems show displacement effects, the position moved by a given steroid being different from that found in the absence of other substances. In partition systems, the movement of the steroid is not affected by the presence of other substances. It was found that 21-hydroxy steroids and 21-dehydro steroids, as well as etienic acids, all moved to characteristic positions on chromatograms alone or in mixture with each other, con-

sistent with chromatographic separations of the partition type. However, to ensure that displacement effects were not influencing the positions moved by the various steroid derivatives during routine chromatographic analyses, one position on the chromatogram always contained a mixture of the steroid alcohol and its derivatives.

Effect of calcium sulphate. Silica gel containing no binder (Merck HF₂₅₄) was compared with silica gel containing calcium sulphate (Merck GF₂₅₄, containing 13% of calcium sulphate) with respect to steroid mobility and separation. In the absence of calcium sulphate, chromatograms streaked badly and steroids did not move in a reproducible manner. The use of mixed-steroid chromatograms indicated that the separations were occurring in part by displacement. It is suggested that the calcium sulphate increased the water content of the stationary phase and thereby aided the conversion of the system into a partition type.

Effect of filter-paper liner. When the inside border of the chromatography jar was lined with sheets of Whatman no. 1 filter paper, solvent moved more rapidly up the thin-layer plate than in the absence of liner. The R_F values were invariably decreased, but ΔR_M values were not significantly altered, indicating that some variation in absolute R_F may be tolerated without altering the ΔR_M values. The spots were circular and compact in the presence or absence of a liner. Results reported in the present paper were obtained in systems without filter-paper liner. Border effects (Stahl, 1959) were occasionally observed on plates developed in unlined jars during the early morning hours. These plates were discarded.

Effect of varying the solvent composition. Fig. 1 shows the effect of varying the solvent composition on the mobilities of cortisol, deoxycorticosterone and their derivatives. The proportions of components of the solvent mixture had a definite effect on the R_M values, but only in the acetone–chloroform–acetic acid system was there a marked change in ΔR_M values. Therefore, although small inaccuracies in measuring out the solvents may influence the R_F values, they would have little or no effect on the ΔR_M values. Because of the continuous variation of mobilities with change in solvent composition, it should be possible to select a solvent mixture that would allow the steroids to move to positions where R_F values could be measured with optimum precision.

ΔR_M values. Table 1 summarizes the ΔR_M values of various natural and synthetic corticosteroids. The values fall within a narrow range for each class of steroid. The ΔR_M values for the conversion of 21-hydroxy steroids into 21-dehydro steroids were the same for 17 α -hydroxy steroids and 17-deoxy steroids; ΔR_M values for the conversion of 21-

hydroxy steroids or 21-dehydro steroids into etienic acids were uniformly more negative for 17α -hydroxy steroids than for 17-deoxy steroids. (These differences probably reflect the increased acidity of the 17-carboxyl group induced by the presence of an adjacent hydroxyl function; Braude & Nachod, 1955.) The increased favourable partition with respect to the aqueous phase resulting from the addition of a 17-hydroxy group to the 17-carboxyl steroid was far greater than predicted for the replacement of a 17α -hydrogen by a 17α -hydroxy group in C_{21} steroids. As shown in Table 1, the ΔR_M values for this change, illustrated by comparing corticosterone and cortisol derivatives, were the same within the limits of error with respect to both 21-alcohols and 21-aldehydes in each solvent system, in contrast with the much more negative ΔR_M values observed when a 17α -hydroxy group replaced the 17α -hydrogen in the corresponding 17β -carboxyl derivatives. That the effects observed are specific for the change from 17-hydrogen to 17-hydroxy group is shown in Table 1, which indicates that the conversion from 11β -hydrogen into 11β -hydroxy group did not affect the R_M values.

In this case, illustrated by the comparison of 11-deoxycortisol and cortisol, ΔR_M values were similar for 21-alcohol, 21-aldehyde and etienic acid for each solvent system. The highly polar acid derivatives listed in Table 1 moved very little or not at all in solvent 5, and reliable ΔR_M values could be obtained only for the etienic acids corresponding to deoxycorticosterone and 11-dehydrocorticosterone.

Effect of oxidation procedures on other steroids. Neither cupric acetate nor periodic acid effected the oxidation of steroids that did not contain the 17-ketol side chain. These included oestradiol, testosterone, 3α -hydroxy-5-androsten-17-one, progesterone, 6β -hydroxypregn-4-ene-3,20-dione, 17α -hydroxypregn-4-ene-3,20-dione, pregna-4,16-diene-3,20-dione and pregnane-3 α ,20 β -diol. However, two steroids containing the 17β -hydroxy-16-oxo grouping, essentially a ring ketol, were attacked by both Cu^{2+} ions and periodate, as shown in Table 2. The ΔR_M values resulting from oxidation of the D ring by cupric acetate were somewhat higher than those obtained for the oxidation of the side chain, but were of the same sign and

Fig. 1. Effects of solvent composition on the mobilities of steroids. Designated solvent mixtures were equilibrated in closed wide-mouth jars for 30 min. before the introduction of the thin-layer plate. Solvent ascended the plate for 100 mm. ●, Cortisol; ▲, 21-dehydrocortisol; ■, $11\beta,17\alpha$ -dihydroxy-3-oxoandro-4-ene-17 β -carboxylic acid; ○, deoxycorticosterone; △, 21-dehydrodeoxycorticosterone; □, 3-oxoandro-4-ene-17 β -carboxylic acid.

Table 1. ΔR_M values for derivatives of 17 β -ketol steroids

Details of experimental procedures and composition of solvent systems are given in the text. For each steroid, the ΔR_M value in each solvent is the mean of six independent determinations.

Solvent	R_M for untreated steroid - R_M for steroid 21-aldehyde					R_M for steroid 21-aldehyde - R_M for steroid etenic acid					R_M for untreated steroid - R_M for steroid etenic acid				
	1	2	3	4	5	1	2	3	4	5	1	2	3	4	5
17 α -Deoxy ketol steroids															
Deoxycorticosterone	+0.26	+0.20	—	—	+0.37	—	+0.18	-0.04	—	-1.29	—	+0.42	—	—	-0.99
Corticosterone	+0.31	+0.27	+0.49	+0.39	+0.39	+0.17	+0.18	+0.04	+0.02	—	+0.35	+0.46	+0.49	+0.41	—
11-Dehydrocorticosterone	+0.42	+0.28	+0.50	+0.40	+0.56	+0.28	+0.25	0.00	0.00	-1.14	+0.33	+0.50	+0.50	+0.40	—
3 β ,11 β ,21-Trihydroxyallopregnan-20-one	+0.34	+0.20	+0.34	+0.22	+0.40	+0.09	+0.20	0.00	0.00	—	+0.43	+0.40	+0.37	+0.22	—
3 β ,21-Dihydroxyallopregnane-11,20-dione	+0.24	+0.22	+0.28	+0.24	+0.28	+0.18	+0.17	0.00	0.00	—	+0.42	+0.38	+0.28	+0.24	—
Mean $\pm$ s.d.	+0.31 $\pm$ 0.07	+0.23 $\pm$ 0.04	+0.40 $\pm$ 0.11	+0.31 $\pm$ 0.09	+0.40 $\pm$ 0.10	+0.18 $\pm$ 0.08	+0.20 $\pm$ 0.03	0.00 $\pm$ 0.03	0.00 $\pm$ 0.01	-1.22	+0.38 $\pm$ 0.05	+0.43 $\pm$ 0.05	+0.41 $\pm$ 0.10	+0.32 $\pm$ 0.10	-0.99
17 α -Hydroxy ketol steroids															
Cortisol	+0.23	+0.25	+0.44	+0.37	+0.53	-1.13	-0.16	-0.64	-0.54	—	-0.88	+0.11	-0.24	-0.16	—
Cortisone	+0.20	+0.21	+0.38	+0.37	+0.49	-1.30	-0.11	-0.62	-0.58	—	-1.06	+0.08	-0.32	-0.29	—
11-Deoxycortisol	+0.27	+0.17	+0.39	+0.31	+0.65	-1.12	-0.01	-0.54	-0.51	—	-0.92	+0.13	-0.27	-0.20	—
Δ^1 -Cortisone	+0.16	+0.23	+0.25	+0.26	+0.52	-1.31	-0.10	-0.68	-0.51	—	-1.15	+0.13	-0.44	-0.25	—
Δ^1 -Cortisol	+0.17	+0.21	+0.25	+0.24	+0.55	-1.24	-0.11	-0.57	-0.40	—	-1.04	+0.10	-0.31	-0.17	—
9 α -Fluorocortisone	+0.18	+0.19	+0.37	+0.15	—	-1.29	-0.08	-0.69	-0.35	—	-1.07	+0.11	-0.32	-0.17	—
3 α ,17 α ,21-Trihydroxy-5 β -pregnan-3-one	+0.40	+0.16	+0.30	+0.24	+0.46	-1.42	-0.03	-0.43	-0.34	—	-1.00	+0.12	-0.12	-0.10	—
17 α ,21-Dihydroxy-5 β -pregnan-3-one	+0.08	+0.15	+0.26	+0.31	+0.70	-1.15	-0.04	-0.45	-0.45	—	-1.08	+0.10	-0.24	-0.13	—
Mean $\pm$ s.d.	+0.21 $\pm$ 0.07	+0.20 $\pm$ 0.03	+0.33 $\pm$ 0.07	+0.28 $\pm$ 0.07	+0.56 $\pm$ 0.09	-1.25 $\pm$ 0.11	-0.08 $\pm$ 0.05	-0.58 $\pm$ 0.08	-0.46 $\pm$ 0.09	—	-1.02 $\pm$ 0.09	+0.11 $\pm$ 0.02	-0.28 $\pm$ 0.09	-0.18 $\pm$ 0.06	—

Table 2. ΔR_M values for 16,17-oxygenated steroids

Details of experimental procedures and composition of solvent systems are given in the text. X, Periodate-oxidation product remained at or near the origin; Y, periodate-oxidation product moved to or near the solvent front.

Solvent	R_M for untreated steroid - R_M for steroid oxidized with cupric acetate					R_M for steroid oxidized with cupric acetate - R_M for steroid oxidized with periodic acid					R_M for untreated steroid - R_M for steroid oxidized with periodic acid				
	1	2	3	4	5	1	2	3	4	5	1	2	3	4	5
9 α -Fluoro-16 α -hydroxycortisone	+0.57	+0.68	+0.69	+0.74	—	-0.42	-0.48	-0.44	-0.52	X	+0.15	+0.20	+0.24	+0.22	X
3 β ,17 β -Dihydroxy-5-androsten-16-one	+0.33	+0.34	+0.34	+0.28	+0.13	-1.86	-1.97	-1.71	-1.74	X	-1.53	-1.63	-1.37	-1.46	X
Oestrone	0.00	0.00	0.00	0.00	0.00	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y
17 β -Oestradiol-16-one	+0.42	+0.67	—	+0.56	+0.35	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y
Triamcinolone	+0.58	+0.75	+0.79	+0.71	—	-0.36	-0.50	-0.58	-0.57	X	+0.14	+0.24	+0.29	+0.06	X
Tramcinolone acetonide	+0.25	+0.33	+0.39	+0.35	+0.42	-1.13	-0.57	-1.00	-0.93	X	-0.90	-0.34	-0.64	-0.58	X

approximately the same magnitude, suggesting that the ΔR_M values obtained are characteristic for the conversion of a ketol function to a *gem*-dione function. The group of steroids containing a ring ketol may be differentiated from that containing a side-chain ketol function by the different effects of periodate on the two classes of steroid. Periodic acid oxidized 17 β -oestradiol-16-one to a derivative that moved with the front in all solvent systems, whereas 3 β ,17 β -dihydroxy-5-androsten-16-one was converted by this treatment into a derivative that showed relatively little movement from the origin, and consequently large negative ΔR_M values with respect to alcohol or aldehyde. Oestriol, as shown in Table 2, was not attacked by cupric acetate, but was oxidized by periodate to a mixture of substances that migrated near the solvent front and yielded a bright-red colour when heated with guaiacol in 50% sulphuric acid. The oxidation of 9 α -fluoro-16 α -hydroxycortisone and 9 α -fluoro-16 α -hydroxy- Δ^1 -cortisol (Triamcinolone) with periodic acid yielded products that had ΔR_M values significantly different from those of 16-deoxy-17 α -hydroxy ketol steroids. The acetone of Triamcinolone yielded an aldehyde with a ΔR_M value relative to the steroid alcohol which was the same as those of the 16-deoxy steroids listed in Table 1. These results are in contrast with the more positive ΔR_M values for the difference between 21-alcohol and 21-aldehyde in the steroids containing a free 16 α -alcohol function. The etienic acid derived from Triamcinolone acetone had more negative ΔR_M values relative to the 21-aldehyde or 21-alcohol than did the 16-deoxy steroids.

DISCUSSION

Certain regular alterations appear in the properties of steroids with modification of structure as measured by spectral shifts (Bladon, Henbest & Wood, 1952) or optical rotation (Barton & Klyne, 1948; Djerassi, 1960). Bush (1961) has shown that changes in the chromatographic behaviour of steroids may similarly be correlated with structure. These regularities depend on the nature and orientation of the substituents on the steroid ring. The analysis presented by Bush is an extension of the thermodynamic derivation due to Martin (1950) and Martin & Synge (1941), based on the behaviour of a solute partitioned between two immiscible solvents. Martin showed that, for two substances A and B differing only by a factor X, the partition coefficients, α_A and α_B , and the difference in chemical potential between A and B, or $\Delta\mu_X$, are related by the equation:

$$\ln \frac{\alpha_A}{\alpha_B} = \frac{\Delta\mu_X}{RT}$$

From this equation and the definition of R_F , Bates-Smith & Westall (1950) derived the relationship:

$$R_M = \log \left(\frac{1}{R_F} - 1 \right)$$

where R_M is a function of the partition coefficient. The difference in R_M , i.e. ΔR_M , is dependent on the nature of the substituents on the steroid nucleus, and in the absence of interactions between them is an additive function of the various groups. The additive nature of this function has been confirmed for a number of non-steroidal compounds (Reichl, 1955; Bush & Hockaday, 1962; Franc & Jokl, 1959) as well as for steroids (Kabasakalian & Basch, 1960) by using paper chromatography. Green & Martinkiewicz (1963) and Martinkiewicz, Green & McHale (1963), in an elegant and thorough study, showed by the use of 'tankless' paper chromatography that ΔR_M values are constant and reproducible for atoms as well as groups.

Although most of the studies involving the ΔR_M function have utilized paper-chromatographic procedures, it has also been possible to correlate the R_M values of steroids obtained on paper and the mobilities of steroids on columns (Kabasakalian & Talmage, 1962). There have been no reports of the application of the ΔR_M concept to thin-layer chromatography of steroids. In the present paper it has been shown that such an application is indeed possible. The ΔR_M values for the conversion of 17 β -ketol steroids into 21-dehydro steroids are similar for the various types of steroids studied in each solvent system. The ΔR_M values for the conversion of C_{21} steroids into the C_{20} acids can be divided into two groups, depending on whether a 17 α -hydroxy group is present. The presence of a hydroxy group α to a carboxyl group has been shown to increase the degree of dissociation of model acids (Braude & Nachod, 1955). The effects of the 17 α -hydroxy group on the chromatographic mobility of the acid relative to the parent C_{21} steroids is consistent with the increased acidity caused by the presence of the adjacent hydroxy group. This conclusion is reinforced by the observation that the replacement of the 11 β -hydrogen by an 11 β -hydroxyl function did not have a similar effect on mobility. It was therefore possible to differentiate between 17 α -hydroxy- and 17-deoxycorticosteroids by chromatography of their etienic acid derivatives. The 21-dehydro corticosteroids and etienic acids containing both 16 α - and 17 α -hydroxy groups showed large deviations from the expected values, probably due to neighbouring-group effects of the ring alcohol functions on the steroid side chain. An attempt to suppress these effects by blocking the 16 α - and 17 α -hydroxy groups by the formation of the cyclic ketol was successful in bringing the ΔR_M of the 21-dehydro

derivative into the same range as those of the 16-deoxy steroids with respect to the 21-alcohol, but the ΔR_M of the etienic acid with respect to either 21-hydroxy or 21-deoxy derivatives was much more negative than those of the 16-deoxy-17-hydroxy steroids or 16,17-dideoxy steroids. These results confirm that neighbouring groups may have a profound effect on the R_M values of reactive groups of steroids. By assuming the validity of Martin's postulates, it should be possible to show that these neighbouring groups affect the mobilities and ΔR_M values in a characteristic and predictable manner.

SUMMARY

1. A procedure is described for the characterization of the steroid 17 β -ketol side chain by comparison of the mobilities of the steroid, its 21-dehydro and etienic acid derivatives by using thin-layer chromatography.

2. A technique for preparing these derivatives on a small scale is described.

3. The difference in R_M , i.e. ΔR_M , between the 21-hydroxy steroids and 21-dehydro steroids was constant for 13 steroids; ΔR_M values for etienic acids were divided into two groups, dependent on the presence or absence of a 17 α -hydroxy group.

4. By using the procedure described, steroids that contained the 17-ketol side chain could be readily distinguished from those that did not contain this grouping.

This work was aided by grants to Dr Abraham White from the National Science Foundation (G-18960), the American Cancer Society (P-68-E), and the National

Institutes of Health, U.S. Public Health Service (CA-03154). The able assistance of Mrs Barbara Jacobson is gratefully acknowledged.

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Biochem. J. (1964) **90**, 527

The Preparation of [6,7- $^3\text{H}_2$]Oestrogens

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(Received 20 May 1963)

It is apparent that radioactive oestrogens of high specific activity would be useful in studies of the metabolism of these hormones. Because of its many advantages (Glascock, 1954; Pearlman, 1957) tritium was chosen as the isotope for this

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purpose. 3-Acetoxyoestra-1,3,5(10),6-tetraen-17-one (I), commonly referred to as Δ^6 -dehydro-oestrone acetate (Pearlman & Wintersteiner, 1940), was catalytically reduced in an atmosphere of tritium-hydrogen gas to form [6,7- $^3\text{H}_2$]oestrone acetate (II) as indicated in Scheme 1; [6,7- $^3\text{H}_2$]oestradiol-17 β (IV) was prepared by the reduction of [6,7- $^3\text{H}_2$]oestrone (III); and [6,7- $^3\text{H}_2$]oestriol (VII) was prepared from compound (III) by a