The mechanical and electrical mechanism can also be readily adapted for automatic operation of a liquid-liquid counter-current distribution apparatus. This only involves rotation of C_1 on N so that it opens S_1 rather earlier in the cycle, for example after A has rotated through about 30°. M_2 is then switched off and the glass units stay in the settling position, i.e. until the two liquid phases have separated, for the period determined by the timer y position. When the timer switches over to the x position, M_2 completes its 360° rotation as described above, A completes its clockwise 90° movement to the first transfer position and its anticlockwise return to the initial position. The upper phase in the Craig units is thus decanted into the upper tube and then passed on to the next unit.

SUMMARY

1. A glass counter-current apparatus suitable for working with solid-liquid systems is described. Provision is made for equilibrating a solute between the two phases, separation of the phases by filtration and transfer of the liquid phase to the next unit.

2. An electrically operated mechanism is described suitable for carrying out the operation cycle automatically.

3. Adaptation of the mechanism to operation of a liquid-liquid counter-current apparatus is also described.

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The Oxidation of Corticosteroids with Sodium Bismuthate

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Lead tetraacetate and periodic acid are the reagents commonly chosen for the selective fission of 1:2-glycols under mild conditions (cf. Jackson, 1944; Criegee, 1948; Johnson, 1951). Both reagents are also capable of cleaving 1:2-ketols, though for substances of this type their effectiveness can vary greatly with the molecular environment of the ketol grouping. The two oxidants differ in their action on α-hydroxy acids, which are cleaved by lead tetraacetate but remain unaffected by periodate. In general, when the effect of either reagent is expected to be the same, lead tetraacetate is taken for reactions in organic solvents, while periodate is preferred for oxidations in aqueous media and for analytical purposes.

Periodate oxidation has proved of great value in elucidating the structures of corticosteroids (cf. Reichstein & Shoppee, 1943), and forms the basis of the analytical determination of 'formaldehydogenic' steroids (Lowenstein, Corcoran & Page, 1946; Daughaday, Jaffe & Williams, 1948; Corcoran, Page & Dustan, 1950), 'acetaldehydogenic' steroids (Butler & Marrian, 1937, 1938; Cox, 1952) and corticosteroids which are converted by periodate to 17-ketosteroids (Talbot & Eitingon, 1944; Fieser, Fields & Lieberman, 1944). The application of lead tetraacetate in this field was less successful: Reichstein (1937) succeeded in converting 11β:17α:21-trihydroxypregn-4-ene-3:20-dione (V) to 11β-hydroxyandrost-4-ene-3:17-dione (XII) with this reagent, but observed that the reaction was sluggish and the yield poor.

A consideration of the mechanism of glycol fission with lead tetraacetate and with periodic acid led Heidt, Gladding & Purves (1945) to postulate certain physical properties required of any oxidant capable of cleaving glycols by the same mechanism. Two oxidants found to have such properties were tervalent silver and sodium bismuthate. The latter reagent, in particular, was shown to effect smooth cleavage of ethylene glycol: quantitative yields of formaldehyde were obtained by shaking a dilute aqueous solution of glycol (at pH $\leqslant$ 4·7) with a large excess of sodium bismuthate. The reaction was complete within 30 min. at room temperature. Rigby (1949, 1950) has independently investigated the action of sodium bismuthate on various simple 1:2-glycols, 1:2-ketols and α-hydroxy acids. The oxidations were performed, in general, with the theoretical amounts of reagent, the medium being either glacial acetic acid or an aqueous solution containing a slight excess of phosphoric acid. The author concluded that in its effect, selectivity and rate of reaction sodium bismuthate closely

resembles periodic acid and lead tetraacetate. In contradistinction to periodate, however, sodium bismuthate was found to cleave α-hydroxy acids.

It appeared to us that this last finding could be exploited for the characterization of the important group of corticosteroids bearing a dihydroxyacetone side chain (type (*d*), Table 1): whereas periodate degrades these only to the α-hydroxy acid stage, oxidation with bismuthate should convert them to 17-ketosteroids, which are relatively easy to characterize and to assay. Furthermore, the insolubility of sodium bismuthate promised to offer technical advantages over the use of periodate, e.g. in permitting the employment of a large excess of the reagent and simple separation of the surplus. Consequently, we have explored the usefulness of the bismuthate oxidation for the characterization of corticosteroids on both the preparative and the micro-analytical scale. Part of this work has been briefly reported in a preliminary communication (Brooks & Norymberski, 1952).

Seven compounds (I–VII, Table 1), representing the six typical corticosteroid side chains (*a*)–(*f*), have been chosen as model substances in this work. The 17:20-diol (VI) was prepared by catalytic hydrogenation of the 17:20-ketol (VII). The 20:21-diol (I) was prepared from 3β:21-dihydroxypregn-5-en-20-one 21-acetate (A; R′ = >CH_2, R″ = >CH.CO.CH_2OAc) by reduction with lithium aluminium hydride. This transformation has been previously effected by the Meerwein-Ponndorf method (Steiger & Reichstein, 1938; Ehrenstein, 1943; Fieser *et al.* 1944): from the difficultly separable mixture of 20-epimers thus obtained, Ehrenstein isolated one of the epimers, which had m.p. 222–229°, $[\alpha]_D - 54°$. From the lithium aluminium hydride reduction we obtained without difficulty a compound of the composition $C_{21}H_{34}O_3$, m.p. 221–224°, $[\alpha]_D - 56°$, identical with Ehrenstein's product, a specimen of which was kindly made available by Dr Ehrenstein.

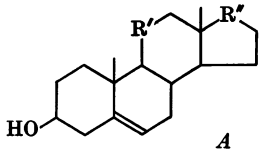
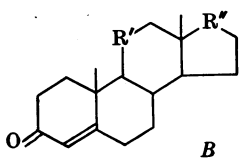
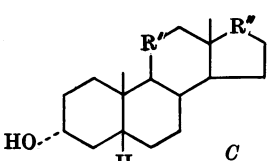
Acetylation of the mother liquors afforded a triacetate, m.p. 182–186°, $[\alpha]_D - 22°$, identical with that prepared from the pure 20:21-diol. The increment in molecular rotation on acetylation (Δ_1) is +81°. Since no vicinal action has been observed between the hydroxyl groups at $C_{(20)}$ and $C_{(21)}$ (Sarett, 1949*b*) the Δ_1 value found for our compound should equal the sum of Δ_1 values for each hydroxyl group. Δ_1 at $C_{(3)}$ for a 5:6-unsaturated 3β-hydroxysteroid is −28° (Barton, 1946), Δ_1 at $C_{(20)}$ is −44° for the α-epimer and +111° for the β-epimer (Klyne & Barton, 1949), and Δ_1 at $C_{(21)}$ is 0° (Sarett, 1949*b*). The increment on acetylation calculated for pregn-5-ene-3β:20α:21-triol is −72° (−28 −44 +0) and for the 20β-epimer +83° (−28 +111 +0). The latter value is in agreement with that found for our product which must therefore be pregn-5-ene-3β:20β-21-triol (I).

The preparative oxidations were performed on the compounds II–VII, with a large excess of sodium bismuthate, in 50% acetic acid. The 20:21-diol (I) was not included in the preparative work since the small quantity of this substance available was reserved for analytical studies. The 17:20-ketol (VII) remained unchanged while the other substances were converted in excellent yields into the products listed in Table 1. The two dihydroxyacetone derivatives IV and V afforded, as was hoped, the corresponding 17-ketosteroids XI and XII. In spite of the large excess of oxidant employed, no effect on any of the nuclear substituents in II–VII was observed.

On the analytical scale, the course and extent of the oxidations were determined by measuring the released formaldehyde and/or 17-ketosteroids. The former was estimated by its colour reaction with chromotropic acid and sulphuric acid (Eegriwe, 1937; cf. MacFadyen, 1945), and the latter were assayed by the Zimmermann reaction (cf. Callow, 1950) with *m*-dinitrobenzene and potassium hydroxide. In order to evaluate the results of the Zimmermann estimations the colorimetric equivalents of the starting materials and of the expected products were determined. The oxidations were performed in the dark with an excess of sodium bismuthate in 5% acetic acid. Under these conditions good recoveries of formaldehyde and/or 17-ketosteroids from substances I–VI were observed, while the 17:20-ketol (VII) did not yield either product. A comparative series of oxidations with periodate revealed, in agreement with the preparative experiments, the difference in the action of the two oxidants on the dihydroxyacetone derivatives IV and V. For the examples of deoxycorticosterone (DOC, II) and cortisone (IV), the quantities of formaldehyde and/or 17-ketosteroids released on oxidation with bismuthate were directly proportional to the quantity of corticosteroid used. Finally the procedure was applied to a urinary extract: in this case it was found necessary to purify the crude non-volatile oxidation product by Girard separation, prior to the estimation of 17-ketosteroids.

The accuracy and reproducibility of the formaldehyde estimation depends on the rigorous exclusion of daylight during the oxidation with sodium bismuthate: the presence of light evidently catalyses the interaction between bismuthate and aqueous acetic acid to give a product which is a chromogen with the chromotropic-acid reagent, with the consequence of gross and irregular increases in the 'blank' values. The effect of light is greatly weakened if propionic acid is substituted for acetic acid in the oxidation medium. Edwards & Kellie (private communication from Dr A. E. Kellie, Courtauld Institute, Middlesex Hospital, London)

Table 1. *Summary of oxidations with sodium bismuthate*

Nuclear type	Corticosteroid R′		Corticosteroid	Corticosteroid R″	Product	Product	Product R″
 *A*	CH_2	I	3β:20β:21-Trihydroxypregn-5-ene (20:21-diol)	CH_2OH | $CHOH$ (*a*) | C...H	VIII	17β-Formylandrost-5-en-3β-ol (not isolated)	CHO | C...H
	CH_2	II	21-Hydroxypregn-4-ene-3:20-dione (deoxycorticosterone) (DOC)	CH_2OH | CO (*b*) | C...H	IX	3-Oxoeti-4-enic acid	COOH | C...H
 *B*	CH_2	III	17α:20β:21-Trihydroxypregn-4-en-3-one (triol)	CH_2OH | $CHOH$ (*c*) | C...OH	X	Androst-4-ene-3:17-dione	CO
	CO	IV	17α:21-Dihydroxypregn-4-ene-3:11:20-trione (cortisone)	CH_2OH | CO (*d*) | C...OH	XI	Androst-4-ene-3:11:17-trione (adrenosterone)	CO
	HO—CH	V	11β:17α:21-Trihydroxypregn-4-ene-3:20-dione (Kendall's compound F)	CH_2OH | CO (*d*) | C...OH	XII	11β-Hydroxyandrost-4-ene-3:17-dione	CO
 *C*	CO	VI	3α:17α:20β-Trihydroxy-pregnan-11-one (17:20-diol)	CH_3 | $CHOH$ (*e*) | C...OH	XIII	3α-Hydroxy-5β-androstane-11:17-dione	CO
	CO	VII	3α:17α-Dihydroxypregnane-11:20-dione (17:20-ketol)	CH_3 | CO (*f*) | C...OH		(Unchanged)	

have developed an improved method for the estimation of formaldehydogenic steroids in which propionic acid is advantageously employed.

EXPERIMENTAL

Melting points were determined on a Kofler stage. Microanalyses are by Weiler and Strauss, Oxford. Alumina for chromatography (Hopkin and Williams) was neutralized by treatment with ethyl acetate overnight followed by exhaustive washing with water, and reactivated at 200°.

Of the corticosteroids I–VII (see Table 1), II (as the acetate) and III were kindly provided by British Schering Ltd., V by Merck and Co. Inc. and VII by N. V. Organon. Hydrolysis of commercial cortisone acetate afforded IV, while I and VI were prepared as described below.

Preparations

Pregn-5-ene-3β:20β:21-triol (I). 3β:21-Dihydroxypregn-5-en-20-one 21-acetate (A; $R' = >CH_2$, $R'' = >CH.CO.CH_2OAc$) was supplied by British Schering Ltd.: 60 mg. was refluxed for 1·5 hr. with $LiAlH_4$ (200 mg.) in ether (20 ml.) and the reaction mixture left overnight at room temperature. The excess of reagent was destroyed with ethyl acetate and the mixture worked up in the usual manner. Crystallization of the crude product from acetone yielded the 20:21-diol (I) (25 mg.) as small plates, m.p. 214–224°. Further crystallization raised the m.p. to 221–224°, $[\alpha]_D - 56°$ in $CHCl_3$: ethanol (5:1, v/v) (*c*, 0·43). (Found: C, 74·9; H, 10·1. Calc. for $C_{21}H_{34}O_3$: C, 75·4; H, 10·2%.) A sample of m.p. 223–225° showed no depression of m.p. on admixture with Ehrenstein's product (see p. 372) of m.p. 226–228°. The mother liquors of I were evaporated to dryness and the residue (30 mg.) acetylated with pyridine and acetic anhydride on the steam bath (30 min.). The product was chromatographed over 2 g. of Al_2O_3. Elution with CCl_4 yielded first a fraction (10 mg.) which crystallized from methanol as leaflets, m.p. 207–209°: this was not further investigated. Continued elution with the same solvent gave a fraction (19 mg.) which crystallized from methanol as needles, m.p. 182–186°, $[\alpha]_D - 22°$ in $CHCl_3$ (*c*, 0·50). (Found: C, 70·8; H, 9·0. Calc. for $C_{27}H_{40}O_6$: C, 70·4; H, 8·8%.) This substance was identical with the product (m.p. 181–185°) of acetylation of the pure 20:21-diol (I). Hydrolysis with 0·5 N-NaOH in aqueous methanol (80%, v/v) afforded pure 20:21-diol (I), m.p. 221–224°.

3α:17α:20β-Trihydroxypregnan-11-one (VI). Hydrogenation of 3α:17α-dihydroxypregnane-11:20-dione (VII) (400 mg.) was effected under the conditions described by Sarett (1949*a*) for the corresponding 3-acetate. The product was acetylated with acetic anhydride in pyridine at room temperature overnight, affording a crude diacetate (480 mg.), m.p. 241–250°. Recrystallization from aqueous acetone gave prisms (321 mg.), m.p. 249–252°: a second crop (86 mg.) had m.p. 235–247°. Hydrolysis of the diacetate (296 mg.) by refluxing with 2 N-KOH in aqueous methanol (90%, v/v) for 1 hr. yielded a product which crystallized from acetone:ether as prisms (161 mg.), m.p. 222–223°. Sarett (1949*a*) found m.p. 220° for VI and m.p. 249–250° for its diacetate.

Preparative oxidations with sodium bismuthate

General procedure. A solution of the steroid (0·14–0·20 m-mole) in 10 ml. of aqueous acetic acid (50%, v/v) is shaken for 30 min. with sodium bismuthate (20 mol. prop.). The reaction mixture is diluted with water (15–20 ml.), treated with sufficient 3 N-KOH to neutralize 75% of the acetic acid, and shaken with benzene (30 ml.). The resulting mixture is filtered with suction: from the filtrate, the benzene layer is separated and the aqueous layer is extracted with portions of benzene (3 × 30 ml.) which have first been used to wash the filter. The combined benzene extracts are washed with water (4 × 20 ml.), each washing being back-extracted with benzene (2 × 5 ml.). The washed extract is evaporated to dryness and the residue purified by a suitable method: examples are given below.

21-Hydroxypregn-4-ene-3:20-dione. The ketol II (57·5 mg., 0·174 m-mole) yielded 55 mg. of a crude crystalline product, m.p. 237–242°. Recrystallization from acetone:light petroleum gave 3-oxoeti-4-enic acid (IX) as dense prisms (43·6 mg.; 79%), m.p. 237–242°. The mother liquor afforded a second crop (6 mg.; 11%) of the same m.p. Sublimation of the product in high vacuum (200°) gave prisms, m.p. 237–241°, $[\alpha]_D + 161°$ in $CHCl_3$ (*c*, 1·00). The mixed m.p. with authentic 3-oxoeti-4-enic acid of m.p. 237–244°, $[\alpha]_D + 159°$ in $CHCl_3$ (*c*, 0·78), prepared from II by periodate oxidation, showed no depression.

17α:20β:21-Trihydroxypregn-4-en-3-one. The triol III (52·2 mg., 0·15 m-mole) yielded 43 mg. of a crude crystalline product, m.p. 163–171°. Recrystallization from acetone: light petroleum gave androst-4-ene-3:17-dione (X) as prisms (36·7 mg.; 85·5%), m.p. 169–172°. The mixed m.p. with authentic androstenedione of m.p. 170–172·5° showed no depression. A portion sublimed in high vacuum (150°) had m.p. 171–173°, $[\alpha]_D + 196°$ in $CHCl_3$ (*c*, 1·61).

17α:21-Dihydroxypregn-4-ene-3:11:20-trione. The dihydroxy acetone IV (56·2 mg., 0·156 m-mole) yielded 47 mg. of a crude crystalline product, m.p. 213–226°. Dissolution in benzene (10 ml.), filtration through Al_2O_3 (1 g.) and elution with 20 ml. more benzene afforded 42·2 mg. (90%) of adrenosterone (XI), m.p. 218–222°. Further elution with benzene (20 ml.) gave 3 mg. (6%) of somewhat less pure material, m.p. 215–225°. Recrystallization from acetone: hexane gave heavy prisms (37·3 mg.), m.p. 224–227°. A sample sublimed in high vacuum (190–200°) had m.p. 223–226°, $[\alpha]_D + 307°$ in $CHCl_3$ (*c*, 1·55). (Found: C, 75·7; H, 8·0. Calc. for $C_{19}H_{24}O_3$: C, 76·0; H, 8·0%). The mixed m.p. with an authentic specimen of adrenosterone, m.p. 223–226°, prepared by chromic-acid oxidation of cortisone (Reichstein, 1936), showed no depression.

11β:17α:21-Trihydroxypregn-4-ene-3:20-dione. The dihydroxyacetone V (51·3 mg., 0·142 m-mole) yielded 42 mg. (98%) of a crude crystalline product, m.p. 195–198°. Dissolution in benzene:ether (9:1, v/v; 30 ml.), filtration through Al_2O_3 (1·5 g.) and elution with a further 30 ml. of the same solvent mixture afforded 40·4 mg. (94%) of 11β-hydroxyandrost-4-ene-3:17-dione (XII) as prisms, m.p. 195–197·5°. Recrystallization from ether:light petroleum gave long needles (28 mg.) of the same m.p., $[\alpha]_D + 219°$ in $CHCl_3$ (*c*, 1·30). (Found: C, 75·1; H, 8·6. Calc. for $C_{19}H_{26}O_3$: C, 75·5; H, 8·7%.) Reichstein (1937) recorded m.p. 190–192° for this compound.

3α:17α:20β-Trihydroxypregnan-11-one. The 17:20-diol VI (51·3 mg., 0·146 m-mole) yielded an oily product. Dissolution in benzene:ether (9:1, v/v; 10 ml.), filtration through Al_2O_3 (1 g.) and elution with 40 ml. of the same solvent mixture afforded 36 mg. (81%) of 3α-hydroxy-5β-androstane-11:17-dione (XIII), m.p. 185–188°. A sample recrystallized from ether:light petroleum had m.p. 188°: it

readily sublimed in high vacuum (165°) giving crystals of the same m.p.; $[\alpha]_D$ +147°, +148° in ethanol (*c*, 0·59, 0·64). (Found: C, 75·1; H, 9·3. Calc. for $C_{19}H_{28}O_3$: C, 75·0; H, 9·3%). Lieberman, Dobriner, Hill, Fieser & Rhoads (1946) gave m.p. 188–189°, $[\alpha]_D$ +96° in ethanol, for this substance. This value, taken in conjunction with that ($[\alpha]_D$ +145°) given by Lieberman *et al.* (1946) for the corresponding acetate, leads to a change of molecular rotation on acetylation (Δ_1 value) of +210°. The standard Δ_1 value recorded by Barton (1946) for 3α-hydroxy-5β-steroids is +83°: for a number of 11-oxopregnanes studied by Sarett (1949*b*) the mean Δ_1 value was +95°.

Further elution of the Al_2O_3 with 50 ml. benzene:ether (1:1, v/v) afforded an oil (5·6 mg.) which crystallized from ether:light petroleum as heavy prisms (4 mg.) of m.p. 220–223° showing no depression of m.p. on admixture with 3α:17α:20β-trihydroxypregnan-11-one of the same m.p. Thus 8% of pure starting material was recovered.

3α:17α-*Dihydroxypregnane*-11:20-*dione*. The ketol VII (26·5 mg., 0·076 m-mole) yielded a crystalline crude product (26 mg.), m.p. 185–200°. Recrystallization from acetone: light petroleum furnished 19·6 mg. (74%) of unchanged material, m.p. and mixed m.p. 199–203°. The mother liquor afforded a further 2·7 mg. (10%) of less pure ketol VII, m.p. 190–200°.

Use of sodium bismuthate oxidations in analytical work

General procedures

Determination of formaldehyde. Method (*i*) (Conway diffusion procedure). Diffusion is carried out according to Hollander, DiMauro & Pearson (1951). After completed diffusion the contents of the inner chambers of the Conway units are transferred to small test tubes and placed in the oven at 100° for 45 min. The solutions are allowed to cool in the dark and are transferred to rectangular cells (2·5 mm. light path) for measurements at 570 mμ. with the Unicam S.P. 350 spectrophotometer. The instrument is set to zero against a processed blank. The time interval between development and measurement is not critical, provided that the solutions are not exposed to daylight: the extinction values at 570 mμ. remain sensibly constant for at least 1 day (cf. MacFadyen, 1945). The results are evaluated from a standard curve (see Fig. 1). *Method* (*ii*) (direct procedure). A sample of the oxidized solution (0·40 ml.) is mixed directly with 0·80 ml. of the chromotropic acid reagent (cf. Rabinovitch, Decombe & Freedman, 1951). Development of colour and all further operations are performed as in the diffusion process.

Determination of 17-*ketosteroids*. The estimations are performed by the procedure of Zygmuntowicz, Wood, Christo & Talbot (1951), a standard of 20 μg. dehydro*epi*androsterone being included with each series. Measurements are made at 520 and 430 mμ. so that a colour correction (Talbot, Berman & McLachlan, 1942; cf. M.R.C. Committee on Clinical Endocrinology, 1951) can be applied. The results are computed, unless otherwise stated, in terms of the corrected extinction values and are expressed as percentage molar equivalents of dehydro*epi*androsterone.

The colour correction of Talbot *et al.* (1942) was empirically evolved for extracts of acid-hydrolysed urines. Its application to pure steroids has the advantage of reducing the chromogenic contribution of the Δ^4-3-keto grouping in the Zimmermann reaction (see Table 2).

Oxidation with sodium bismuthate. Stock solutions of steroids in absolute ethanol are stored in the dark. Appropriate samples are evaporated in a stream of N_2, and finally *in vacuo*, in 125 × 16 mm. test tubes. Each residue is dissolved in 2·0 ml. of aqueous acetic acid (5%, v/v), 5 mg. of sodium bismuthate (A.R.) is added and the tube shaken for 30 min. with protection from daylight. The mixture is centrifuged and 0·40 ml. samples are taken for the estimation of formaldehyde and/or 17-ketosteroid. The latter determination is performed either (*a*) on the sample used for the estimation of formaldehyde by method (i) by extracting (with $CHCl_3$: methanol, 1:1) the residue remaining in the outer chamber of the Conway unit after completed diffusion, or (*b*) on a separate sample which is evaporated to dryness in a vacuum desiccator over KOH.

Oxidation with periodic acid. Measured quantities of steroids are transferred into glass-stoppered test tubes (approx. capacity 10 ml.) as described above. Oxidations with periodate are performed by the procedure of Hollander *et al.* (1951). From each oxidized solution one sample of 0·40 ml. is taken for the estimation of formaldehyde by the diffusion procedure (method (i)). The remaining volume (1·60 ml.) is extracted with ethylene dichloride (4·0 ml.; purified by shaking with conc. H_2SO_4, washing with water, aqueous Na_2CO_3 and water, and redistilling) by mechanical stirring for 4 min.: the phases are separated by centrifuging and the aqueous layer is removed with filter paper. The extract is washed successively (by shaking, centrifuging and separating) with 2N-NaOH (1 ml.) and water (0·5 ml.), and is filtered through a small paper. From the filtrate two samples of 1·0 ml. are taken and evaporated to dryness: the residues are assayed for 17-ketosteroids.

Colorimetric equivalents of pure steroids in the Zimmermann reaction

For a single determination, 20–40 μg. of each 17-ketosteroid and 100–200 μg. of each other steroid were taken. The results were calculated from both the corrected and the uncorrected extinction values and are given in Table 2. A few comparative data quoted by other authors are also recorded: strict agreement cannot be expected in view of differences in the colorimetric technique employed.

Influence of light on the reaction between sodium bismuthate and aqueous acetic acid

These experiments were carried out with the collaboration of Miss A. T. Sermin. In preliminary experiments no precautions were taken to exclude daylight during the oxidation of corticosteroids with sodium bismuthate. Under these conditions, the subsequent determinations of formaldehyde were occasionally vitiated by irregular increases in the 'blank' values. The prime cause of the interference was eventually identified as the light-catalysed reaction between aqueous acetic acid and sodium bismuthate. The relevant experimental results are summarized in Table 3.

Bismuthate oxidations of DOC (II) and cortisone (IV) at varying concentrations

Oxidations were carried out by the general procedure with various concentrations (8–64 μg./ml.) of cortisone and DOC. Estimations of formaldehyde and of 17-ketosteroids

Table 2. *Colorimetric equivalents in the Zimmermann reaction*

(Extinctions are expressed as a percentage of that obtained with 1 molecular proportion of dehydro*epi*androsterone (DHA).)

Steroid	Extinctions (% of that for DHA): Measured at 520 and 430 mμ. and corrected	Measured at 520 mμ. only: This paper	Measured at 520 mμ. only: Other data	Ratio $\frac{E_{520}}{E_{430}}$
20:21-Diol (I)	1	4	—	0·8
DOC (II)	10	28	26*	0·8
Triol (III)	13	28	—	0·9
Cortisone (IV)	1	15	25*	0·6
Compound F (V)	3	18	19*	0·7
17:20-Diol (VI)	2	3	—	1·0
17:20-Ketol (VII)	25	27	—	1·6
3-Oxoeti-4-enic acid (IX)	9	22	—	0·8
Androst-4-ene-3:17-dione (X)	118	140	127†	1·5
Adrenosterone (XI)	97	111	—	1·5
11β-Hydroxyandrost-4-ene-3:17-dione (XII)	69	85	—	1·4
3α-Hydroxy-5β-androstane-11:17-dione (XIII)	124	123	123†	2·3

* Private communication from Dr A. E. Kellie. † Wilson (1950).

Table 3. *Blank values in the estimation of formaldehyde*

(Aqueous acetic or propionic acid, 2·0 ml. and $NaBiO_3$, 5 mg. in each experiment; 0·40 ml. samples taken after shaking.)

Solvent	Concn. (%, v/v)	Shaking time (hr.)	Illumination	Extinction at 570 mμ.*
Aqueous acetic acid stored without precautions to exclude light	1·25	1·5	No precautions to exclude light	0·06–0·3
	5	1·5		0·14–0·6
	1·25	1·5	Light excluded	0·035–0·045
	5	1·5		0·09–0·10
	25	1·5		0·22–0·23
Aqueous acetic acid stored in dark	5	0·5	Light excluded	0·045–0·055
	5	0·5	Electric light	0·065–0·070
	5	0·5	Sunlight	1·38–1·42
Aqueous propionic acid stored in dark	5	0·5	Light excluded	0·035–0·045
	5	0·5	Sunlight	0·085–0·095

* Water as blank.

were performed by the two alternative methods, agreement between which is good (Fig. 1). The diffusion procedure (i) is generally to be preferred over the direct method (ii), but the latter is conveniently rapid and appears from these and other experiments to be applicable when pure steroids are being estimated.

Oxidation of cortical steroids and related substances with bismuthate and with periodate

Oxidations of compounds I–VII (50–100 μg./ml.) were performed by the general procedures. The results are summarized in Table 4.

Recovery of formaldehydogenic and 17-ketogenic steroids following addition of triol (III) to a urine extract

These experiments were carried out with the collaboration of Miss A. T. Sermin. A pooled-urine specimen (3·5 l.) was adjusted to pH 1 with HCl and extracted with 1·2 l. $CHCl_3$. The extract was washed successively with 0·1 N-NaOH (2 × 50 ml.) and water (2 × 50 ml.), each washing being back-extracted with $CHCl_3$ (50 ml.). The combined $CHCl_3$ extracts were evaporated *in vacuo* under a stream of N_2 and the residue dissolved in 5% acetic acid (40·0 ml.). Samples of this solution were taken for the following estimations: (1) estimation of formaldehydogenic steroids by method (i) gave $26·2 \times 10^{-8}$ moles/ml.; (2) as in (1) after addition of $6·66 \times 10^{-8}$ moles/ml. of triol III gave $32·5 \times 10^{-8}$ moles/ml., the increase of $6·3 \times 10^{-8}$ moles/ml. represents a recovery of 95% of the triol III; (3) estimation of 17-ketosteroids preceded by separation with Girard T reagent (cf. Pincus & Pearlman, 1941; the procedure was modified by adjusting to pH 7 with phosphate-citrate buffer prior to extraction of the Girard complex) gave $1·75 \times 10^{-8}$ molar equivalents of dehydro*epi*androsterone/ml.; (4) as in (3) but after oxidation with sodium bismuthate by the general procedure gave $4·6 \times 10^{-8}$ molar equivalents of dehydro*epi*androsterone/ml.; (5) as in (4) but after addition of $6·66 \times 10^{-8}$ moles/ml. of triol III gave $10·25 \times 10^{-8}$ molar equivalents of dehydro*epi*androsterone/ml., the increase of $5·65 \times 10^{-8}$ molar equivalents/ml. represents a recovery of 71% of the triol III (see Table 4).

DISCUSSION

The evidence presented here demonstrates the usefulness of sodium bismuthate in the selective oxidative fission of the corticosteroidal side chain for preparative as well as for analytical purposes. The analytical procedure permits the combined estimation of formaldehydogenic and '17-ketogenic' steroids on one sample containing as little as 10 μg. corticosteroid and affords a simple means of differentiating and estimating three groups of corticosteroids: (i) formaldehydogenic, non-ketogenic (types *a* and *b*); (ii) formaldehydogenic and ketogenic (types *c* and *d*); (iii) ketogenic, non-formaldehydogenic (type *e*). A further differentiation between the various side-chain types can be achieved with the aid of the Porter-Silber reaction for dihydroxyacetone derivatives (*d*) (Porter & Silber, 1950), or by employing analytical methods based on the reducing properties of types (*b*) and (*d*) (Talbot, Saltzman, Wixom & Wolfe, 1945; Heard & Sobel, 1946), or by estimating 17-ketosteroids formed from types (*c*) and (*e*) on oxidation with periodate (Talbot & Eitingon, 1944; Fieser *et al.* 1944).

We find our analytical method applicable to urinary extracts: however, the lack of a means of preparing satisfactory extracts has so far severely limited the value of such applications. For the estimation of urinary corticosteroids of the '17-ketogenic' group ((ii) and (iii) above) we prefer a method in which the problems of hydrolysis and extraction are avoided by carrying out oxidation *in situ* with sodium bismuthate (Norymberski, 1952; Norymberski, Stubbs & West, 1953).

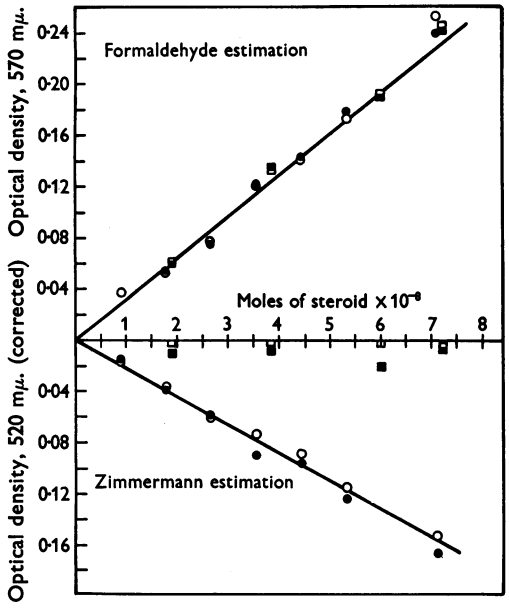


Fig. 1. Bismuthate oxidations of DOC (II) and cortisone (IV).
Upper graph. Formaldehyde estimation method

	(i)	(ii)
DOC	□	■
Cortisone	○	●

Lower graph. Zimmermann estimation method

	(*a*)	(*b*)
DOC	□	■
Cortisone	○	●

SUMMARY

1. Six corticosteroids representing five different side-chain types have been treated with sodium bismuthate in 50 % acetic acid. Five compounds were oxidized in excellent yields, whilst a 17-hydroxy-20-ketone remained unchanged.

2. An analytical procedure has been developed based on the estimation of formaldehyde and/or 17-ketosteroids following the oxidation of corticosteroids with sodium bismuthate. The method has

Table 4. *Results of periodate and bismuthate oxidations*

	Recoveries of products in moles %					
	Periodate oxidations			Bismuthate oxidations		
		17-Ketosteroid as DHA			17-Ketosteroid as DHA	
Steroid	Formaldehyde	Found	Expected*	Formaldehyde†	Found‡	Expected*
20:21-Diol (I)	88	11	—	114	7	—
DOC (II)	105	6	0§	93	5	9
Triol (III)	98	115	118	111	116	118
Cortisone (IV)	90	16	0§	98	98	97
Compound F (V)	93	11	0§	91	54	69
17:20-Diol (VI)	12	122	124	8	144	124
17:20-Ketol (VII)	6	34	25	9	30	25

* Based on the colorimetric equivalents (Table 2) of the expected products.
† Determined by method (i).
‡ Determined by method (*b*).
§ Acidic products eliminated during working up.
— = Not measured.

been applied to a urinary extract to which 17α:20β: 21-trihydroxypregn-4-en-3-one (III) was added: reasonable recoveries of formaldehyde and 17-ketosteroid formed from III were obtained.

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Studies on the Proteins of Fish Skeletal Muscle

2. ELECTROPHORETIC ANALYSIS OF LOW IONIC STRENGTH EXTRACTS OF SEVERAL SPECIES OF FISH

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The comparative biochemistry of skeletal-muscle proteins has so far attracted only occasional and incidental attention. From the published work it is possible to make a few comparisons which, however, largely relate either to the fibrillar proteins or particular enzymes of a few types of animal muscle, e.g. rabbit, frog, carp, snail, rat, mouse and insect muscle. In particular, the electrophoretic properties of the fibrillar proteins from different animals are rather similar (Weber & Portzehl, 1952; Hamoir, 1952). Of the proteins which are extractable with dilute salt solutions, only those from frog (Jacob, 1945; Dubuisson & Jacob, 1945), rabbit (Jacob, 1947; Bosch, 1951), carp (Hamoir, 1951) and human (Haan, 1952) muscle have been studied electrophoretically. The derived diagrams show some marked differences between different animals, but the results have been obtained under