and extracted three times with 75-ml. volumes of ether. The combined ether–benzene solution was washed with dilute potassium carbonate solution, water and dried over sodium sulfate. Evaporation of the solvent yielded 2.10 g. of semi-crystalline residue which was crystallized from methylene chloride–Skellysolve B; yield 1.80 g. (77%), m.p. 151–154°. The analytical sample melted at 153–154°, $[\alpha]^{23}D$ +89° ($CHCl_3$).

Anal. Calcd. for $C_{19}H_{26}O_2$: C, 79.68; H, 9.15. Found: C, 79.93; H, 9.10.

9(11)-Dehydrotestosterone Propionate (IIb).—A solution of 0.3 g. of 9(11)-dehydrotestosterone in 2 ml. of dry pyridine was treated with 2 ml. of propionic anhydride and allowed to stand at 26° for 22 hours when it was poured into 25 ml. of water and the mixture stirred for 2 hours at room temperature. The crystalline product was recovered by filtration, washed with water and dried; yield 0.34 g., m.p. 112°. The analytical sample prepared by recrystallization from dilute methanol melted at 114°, $[\alpha]^{23}D$ +63° ($CHCl_3$).

Anal. Calcd. for $C_{22}H_{30}O_3$: C, 77.14; H, 8.83. Found: C, 77.22; H, 8.93.

9(11)-Dehydrotestosterone Benzoate (IIc).—A solution of 250 mg. of 9(11)-dehydrotestosterone in 30 ml. of benzene was distilled to remove 18 ml. of benzene and cooled to 26°. The dry benzene solution of steroid was treated with 0.32 ml. of dry pyridine and 0.32 ml. of benzoyl chloride and allowed to stand at 26° for 5 hours during which time pyridine hydrochloride separated from the solution. The mixture was stirred with 25 ml. of water and extracted with ether. After washing the extract with dilute hydrochloric acid, dilute sodium hydroxide solution and water, the solution was dried over sodium sulfate and the solvent evaporated *in vacuo*. The residue was recrystallized from absolute methanol to yield 270 mg. of benzoate, m.p. 175–177°, $[\alpha]^{23}D$ +113° ($CHCl_3$).

Anal. Calcd. for $C_{26}H_{30}O_3$: C, 79.96; H, 7.74. Found: C, 79.73; H, 7.63.

9(11)-Dehydrotestosterone β-cyclopentylpropionate (IId) was prepared from 250 mg. of 9(11)-dehydrotestosterone and β-cyclopentylpropionyl chloride by the procedure described above for the benzoate. The product was obtained by crystallizing from dilute methanol; m.p. 96°, yield 255 mg., $[\alpha]^{23}D$ +58° ($CHCl_3$).

Anal. Calcd. for $C_{27}H_{38}O_3$: C, 78.98; H, 9.33. Found: C, 78.94; H, 9.21.

4,9(11)-Androstadiene-3,17-dione (IV) by the *trans*-Elimination of the Elements of Water.—11β-Hydroxy-4-androstene-3,17-dione (III) (5.0 g.) suspended in 500 ml. of benzene and 200 ml. of ether was treated with 100 ml. of water and 200 ml. of concentrated hydrochloric acid. The two-phase mixture was stirred vigorously and heated at reflux for 18 hours, the layers separated, and the aqueous portion extracted three times with 100-ml. portions of a 50:50 mixture of ether–benzene. The combined ether–benzene solution was washed with water, dilute sodium carbonate, water and the solvent evaporated to yield 4.33 g. of crystalline residue, m.p. 190–200°. Recrystallization from methylene chloride–Skellysolve B mixture gave 4.06 g. (87%) of 4,9(11)-androstadiene-3,17-dione, m.p. 202–204°, $[\alpha]^{23}D$ +221° ($CHCl_3$), λ_{max}^{alc} 240 mμ (*E* 16,925).[4]

Anal. Calcd. for $C_{19}H_{24}O_2$: C, 80.24; H, 8.51. Found: C, 80.34; H, 8.41.

4,9(11)-Androstadiene-3,17-dione (IV) by Chromic Acid Oxidation of 9(11)-Dehydrotestosterone.—A solution of 280 mg. of 9(11)-dehydrotestosterone (IIa) in 10 ml. of glacial acetic acid was treated with a mixture of 0.14 g. of chromium trioxide, 0.3 ml. of water and 10 ml. of glacial acetic acid at room temperature for two hours. Excess oxidant was destroyed by the addition of 4 ml. of methanol after which the reaction mixture was poured into 50 ml. of water. The product was extracted several times with a 50–50 mixture of ether–benzene, the solution washed with water, dilute sodium hydroxide solution, water and dried over sodium sulfate. Evaporation of the solvent left a white solid residue of 250 mg. which was crystallized from methylene chloride–Skellysolve B to yield 180 mg. (64%) of 4,9(11)-androstadiene-3,17-dione, m.p. 203–205°, identical with the product prepared as described above.

3-(N-Morpholinyl)-3,5-androstadiene-11,17-dione (VI).—Three grams of adrenosterone (V), 40 ml. of Skellysolve C, 6.96 ml. (8 equivalents) of dry morpholine and 40 mg. of *p*-toluenesulfonic acid was stirred and heated at reflux for 3.5 hours. The water of reaction was removed continuously during this period by means of a water trap placed between the reaction flask and the condenser. The mixture was concentrated to dryness *in vacuo* and the residue crystallized from acetone in prisms; m.p. dec. above 230°, $[\alpha]D$ +26° ($CHCl_3$), λ_{max}^{ether} 269 mμ (*E* 21,300).

Anal. Calcd. for $C_{23}H_{31}NO_3$: C, 74.75; H, 8.46; N, 3.79. Found: C, 74.86; H, 8.50; N, 4.12.

The infrared spectrum in Nujol showed absorption for 17-ketone, 1730 $cm.^{-1}$; 11-ketone, 1692 $cm.^{-1}$.

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(4) ADDED IN PROOF.—These data are in close agreement with those reported by S. Bernstein, *et al.*, ref. 3. This compound has now been treated with pyrrolidine to yield 3-(N-pyrrolidyl)-3,5,9(11)-androstatrien-17-one, m.p. dec. above 157°, $[\alpha]D$ −139° ($CHCl_3$); calcd. for $C_{23}H_{31}NO$: C, 81.85; H, 9.26; N, 4.15. Found: C, 82.30; H, 9.36; N, 4.33. Treatment of this C_3-pyrrolidine derivative with lithium aluminum hydride, as previously described for the reduction of 3-(N-pyrrolidyl)-3,5-androstadiene-11,17-dione, ref. 1, and subsequent hydrolysis gave a 90% yield of 9(11)-dehydrotestosterone, m.p. 151–152°; admixture with the compound IIa prepared as described above gave no m.p. depressions.

Purification of Corticotropin

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The use of cellulose for the purification of corticotropin was reported first by Payne, Raben and Astwood.[1] These workers achieved a tenfold concentration of a crude extract of hog anterior pituitary powder. Subsequently a cellulose derivative, oxycellulose, was found[2] to be superior to cellulose yielding a forty-fold purification by a batchwise adsorption technique. This adsorbent has been used subsequently by other workers[3–5] for the purification of hog and sheep pituitary extracts with similar results. In our laboratory experiments with other cellulose derivatives containing an acidic functional group such as cellulose acid citrate and sodium cellulose acid phosphate have shown that these cellulose derivatives also are effective adsorbents for beef corticotropin. Carboxymethyl cellulose appeared less effective. These findings support the view[2] that the cation-exchange properties are important in the fractionation of corticotropin by oxycellulose. However, the remarkable specificity of these substances for corticotropin when compared with common ion exchange materials as well as the use of exchange groups with markedly different dissociation constants indicates that other properties of the substituted cellulose derivatives are important for this fractionation. One possible explanation for this specificity may be the spatial arrangement of exchange groups of each substance such that the formation of a complex

(1) R. W. Payne, M. S. Raben and E. B. Astwood, *J. Biol. Chem.*, **187**, 719 (1950).

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(3) N. G. Brink, F. A. Kuehl, Jr., J. W. Richter, A. W. Bazemore, M. A. Meisinger, D. E. Ayer and K. Folkers, *ibid.*, **74**, 2120 (1952).

(4) C. H. Li, *ibid.*, **74**, 2124 (1952).

(5) A. W. Bazemore, J. W. Richter, D. E. Ayer, G. Finnerty, N. G. Brink and K. Folkers, *ibid.*, **75**, 1949 (1953).

which is stabilized further by hydrogen bonding and by van der Waals attractions is possible. The relatively low yield of purified product may be considered as further evidence for such a complex.

This work was carried out with corticotropin of beef origin. Beef glands contain a much lower concentration of corticotropin than pork, and best results were obtained using methanol–acetic acid extraction of defatted glands[5] for the preparation of the crude acid extract which was usually obtained at a potency of 0.8–1.2 U.S.P. units per mg. Rapid freezing of the glands after removal was necessary for good results. The yield and potency of corticotropin from various sources is shown in Table I.

TABLE I

YIELD AND POTENCY OF CORTICOTROPIN FROM 1 KG. OF FRESH GLANDS

Source	Defatted gland, wt. (g.)	Crude acid extract Wt. (g.)	Crude acid extract Potency, μ/mg.	Oxycellulose purified Wt. (mg.)	Oxycellulose purified Potency, μ/mg.	Ref.
Sheep	..	..	.....	200	30–50	4
Pork	150	18	2–3	450	84	5
Beef	185	35	0.7–1.2	700	20–30	

Although beef glands yield corticotropin of lower potency and in smaller quantity than either sheep or pork glands, the yield and potency are such that this source of corticotropin could be utilized if necessary. Although beef corticotropin purified by the oxycellulose procedure is undoubtedly different in some aspects from either sheep or pork corticotropin,[4] the applicability and specificity of the cellulose procedures with corticotropin from these sources is evidence that basic groups of similar pK and spatial arrangement are present in each of these preparations. That these groups are identical and a requisite of activity is a further, but more speculative, conclusion.

TABLE II

FRACTIONATION OF BEEF CORTICOTROPIN USING CELLULOSE DERIVATIVES

Sample	Adsorbent	Cellulose derivative, mg. per g. of crude	Stirring time, hr.	Acid extract,[a] μ/mg.[b]	Purified product, μ/mg.	Activity recovery, %
Lot 1	Oxycellulose	193	4	0.75	18	52
	Cellulose acid citrate[6]	250	4	.75	20	44
	Oxycellulose	200	2	.75	25	46
	Oxycellulose	161	24[c]	.75	20	57
Lot 2	Cellulose acid citrate	250	3.5	1.1	30	46
	Oxycellulose	114	2.5	1.1	30	19
	Cellulose acid phosphate[6]	240	3.5	1.1	14	20
Lot 3	Oxycellulose	250	5	0.8	15	..
	Cellulose acid citrate	500	6	.8	11	29
Lot 4	Carboxymethyl cellulose[7]	50	20[d]	.75	19	5

[a] The acetic acid extract was prepared by the method described in ref. 5. [b] U.S.P. units, modified ascorbic acid depletion method.[8] [c] No Tween 20 added. [d] The sodium salt was converted to the free acid form prior to use. The activity was eluted with ammonium hydroxide.

(6) Kindly supplied by Tennessee Eastman Co.

(7) Received from P. D. Welldon, Hercules Powder Co. The product was rendered insoluble in water by partial internal lactonization which was obtained by drying this material at 100° before use.

The experimental procedure consisted in slurrying a dilute solution of the crude acid extract with the cellulose derivative being studied for 2–4 hours in the presence of a surfactant. Using 0.1% of a non-ionic polyoxyethylene sorbitan derivative (Tween-20) the potency and yield of product was similar to that obtained by the published procedures in which a slurry time of 24–40 hours is used. The results obtained with the various cellulose derivatives are shown in Table II.

Experimental

Preparation of Defatted Gland Tissue.—Frozen beef pituitary glands (1 kg.) were partially thawed and ground using a Hobart meat grinder. The ground tissue was slurrried with 4 l. of acetone. The slurry was allowed to settle and the upper phase decanted. The solid residue was slurried and decanted with two additional 2-l. portions of acetone followed by three 2-l. portions of methanol and finally filtered, washed with ether and dried yielding 185 g. of defatted gland tissue. This material may be stored at room temperature for reasonable periods with no detectable loss of activity.

Preparation of Crude Acid Extract.—Defatted beef gland tissue (100 g.) was stirred with 900 ml. of methanol and 600 ml. of acetic acid at reflux temperature for two hours in a closed apparatus protected from moisture by a drying tube. At the end of this time the mixture was centrifuged and the decantate collected. The residue was washed with 40% acetic acid–methanol and the washings combined with the decantate. The crude acid extract was obtained by adding an equal volume of anhydrous ethyl ether to the decantate, filtering, washing with ether and drying, wt. 35 g., potency 0.7–1.2 u. per mg.

Cellulose Purification.—Crude corticotropin was dissolved in sufficient water to make a 2.5% solution. Tween-20 (1 mg. per ml.) was added and the mixture slurried at room temperature with the required amount of cellulose derivative (usually 20–25% of the weight of the crude) using a mechanical stirrer for 2–4 hours. The mixture was filtered and washed with 0.1 N acetic acid until the washings gave a negative biuret test taking care to slurry the mixture well with each wash. Usually three or four washes of 10 ml. for each gram of cellulose derivative were required. The washed adsorbate was slurried for one-half hour with two successive portions of 0.1 N hydrochloric acid (10 ml. total per g. of adsorbate). The combined eluate was neutralized to pH 3.0 by slurrying with strong base ion exchange resin (such as IRA-400) as the carbonate and lyophilized. The dried products are stable at room temperature when stored under moisture free conditions.

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A Method for Preparing Codeinone

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In the course of preparing various glycosides of codeine[1] for biological investigations, the consistently poor yields induced us to examine the

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