[JOINT CONTRIBUTION FROM THE INSTITUTO DE QUÍMICA, UNIVERSIDAD NACIONAL AUTÓNOMA DE MÉXICO AND THE RESEARCH LABORATORIES OF SYNTEX, S. A.]

Steroids. XIII.[1] Reaction of α,β-Unsaturated Steroid Ketones with Benzylmercaptan. Thioenol Ether Formation and 1,4-Addition

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Pyridine hydrochloride has proved to be an excellent catalyst for the conversion of Δ^4-3-ketosteroids (I) with benzyl mercaptan to the corresponding 3-benzylthioenol ethers (II) in the presence of such sensitive carbonyl-containing groupings as the ketol side chain and the 17α-hydroxy-20-ketone moiety characteristic of the adrenal corticosteroids and the spiroketal system of the sapogenins. All of the thioenol ethers were desulfurized to $\Delta^{3,5}$-dienes (III) and further hydrogenated to 3-desoxyallosteroids (IV). Lithium aluminum hydride reduction of 3-benzylthioenol ethers containing a 20-keto group was shown to yield primarily the 20β-hydroxy isomer. 1,4-Addition of benzyl mercaptan to an α,β-unsaturated ketone system is possible if steric factors do not interfere. In the case of 16-dehydroprogesterone (VIII) it has been possible to accomplish thioenol ether formation and 1,4-addition separately or concurrently, depending on reaction conditions. Both 16-sulfoxidobenzyl steroids and 3-benzylsufoxidoenol ethers (XVI) have been prepared by oxidation of the corresponding thio derivatives. While the sulfoxidoenol ether (XVI) proved to be resistant toward acid hydrolysis, it could be reconverted to the acid-labile thioenol ether (II) by lithium aluminum hydride reduction.

With the exception of the ring-A aromatic estrogenic hormones, a Δ^4-3-keto grouping in ring A appears to be essential for optimal physiological activity in the steroid hormone series (androgenic, cortical and progestational hormones). In the synthesis of these substances, the α,β-unsaturated keto moiety is usually introduced as the last step since reactions in other parts of the molecule almost invariably affect this rather labile carbonyl system. Only very few procedures have been developed for blocking the intact Δ^4-3-keto function, and enol ether (or ester) formation appears to be one of the most useful. Thus recently[2] a synthesis of testosterone (If) from Δ^4-androstene-3,17-dione (Ie) was developed, in which the latter was converted into its thioenol ether IIe, the C-17 carbonyl group reduced and the resulting thioenol ether IIf hydrolyzed to the desired hormone If. This sequence of reactions clearly demonstrated that the thioenol ethers are as useful as protecting groups as the corresponding oxygen analogs V. From a general synthetic standpoint, the thio derivatives have the additional advantage that hydrogenolysis produces desoxy derivatives, which at times are quite difficult to prepare by other procedures. The present investigation was concerned with a detailed study of the scope of the reaction of benzylmercaptan (particularly desirable because of well crystallized derivatives) with steroidal ketones, both in regard to reaction conditions and versatility as far as ability to operate in the presence of other sensitive groupings is concerned. Special emphasis was placed on pregnane derivatives because of their intimate connection with synthetic problems in the corticosteroid series.

In the presence of acid catalysts, notably zinc chloride, mercaptans react with saturated as well as Δ^4-3-ketosteroids with formation of mercaptoles. This is true of carbonyl groups at C-3,[3,4,5] C-7,[4,4a] C-12,[4] C-17[4,6,7,8] and C-20.[8] Occasionally, depending on the reaction conditions, thioenol ethers are also formed in the presence of zinc chloride[2,3,8] or *p*-toluenesulfonic acid.[2,9] 1,4-(or 3,4)-Addition of benzyl mercaptan to the Δ^4-3-keto system, though attempted,[4] has failed. With the exception of bile acids[3,4,5] no steroids have been investigated where other substituents in the molecule might react with mercaptans under a given set of conditions.

Since most of the above-mentioned acid catalysts appeared to be too drastic to be used with acid-labile substituents, a search was made for a more suitable condensing agent. Pyridine hydrochloride in benzene solution has now proved to be the catalyst of choice. It had been employed once[2] with fair success in the preparation of a 3-(β-hydroxyethyl)-thioenol ether. When tested with benzyl mercaptan and three Δ^4-3-ketosteroids (Ie, If, Ig), whose benzylthioenol ethers (II) are already known,[2] excellent yields of the corresponding thioenol ethers (IIe, IIf, IIg) were realized even in the presence of a large excess of mercaptan, which presumably would favor mercaptole formation.[4] When applied to progesterone (Ia), the thioenol ether IIa was readily obtained without observing any reaction at C-20 (in contrast to zinc chloride[8]). The structure of the enol ether was proved by desulfurization to the previously undescribed $\Delta^{3,5}$-pregnadien-20-one (IIIa) and thence by reduction to the known allopregnan-20-one (IVa). The mildness and general applicability of this condensing agent was further demonstrated in the preparation of the benzylthioenol ethers of desoxycorticosterone acetate (IIb)[10] and 17α-hydroxyprogesterone (IIc) with their labile carbonyl-containing substituents at C-17. As was to be expected, the spiroketal side chain characteristic of steroidal sapogenins was unattacked as exemplified by the conversion of Δ^4-22-isospirosten-3-one[11] (Id) *via* its thioenol ether IId and diene IIId

(1) Paper XII, Djerassi, Rosenkranz, Iriarte, Berlin and Romo, THIS JOURNAL, **73**, 1523 (1951).

(2) Rosenkranz, Kaufmann and Romo, *ibid.*, **71**, 3689 (1949).

(3) Bernstein and Dorfman, *ibid.*, **68**, 1152 (1946).

(4) Hauptmann, *ibid.*, **69**, 562 (1947).

(4a) Ralls, Dodson and Riegel, *ibid.*, **71**, 3320 (1949).

(5) Jones, Webb and Smith, *J. Chem. Soc.*, 2764 (1949).

(6) Norymberska, Norymbersky and Olalde, THIS JOURNAL, **70**, 1256 (1948).

(7) Levin and Thompson, *ibid.*, **70**, 3140 (1948).

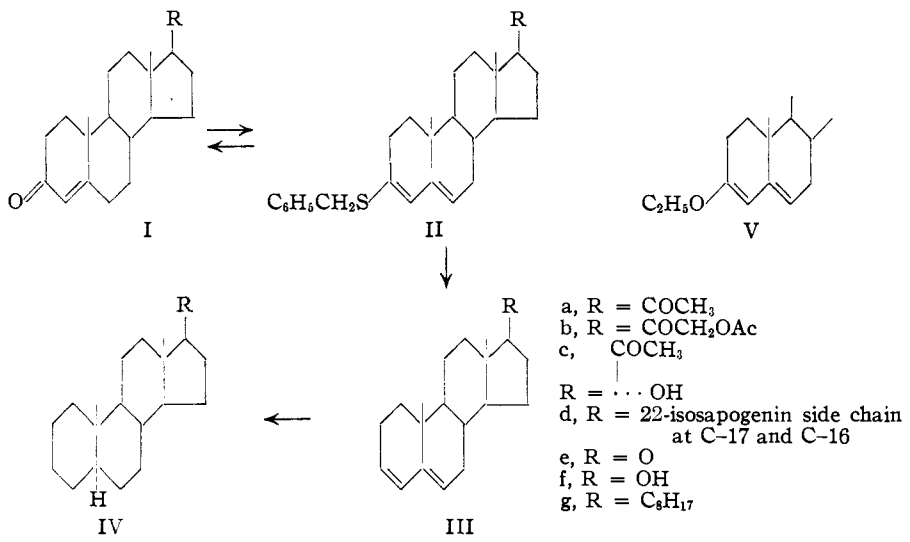
(8) Jeanloz, *ibid.*, **72**, 2281 (1950).

(9) Hungarian Patent 135,687, *C. A.*, **44**, 4047 (1950).

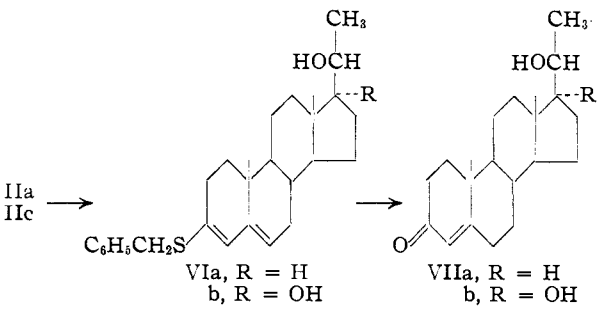
(10) The desulfurization product of IIb, $\Delta^{3,5}$-pregnadien-21-ol-20-one 21-acetate (IIIb), though fully consistent with its ascribed structure as far as elementary analysis, ultraviolet spectrum and conversion to the known desoxyallo ketone IVb are concerned, showed an abnormally high positive rotation. This was also true of a sample of IIIb prepared by another method (see Experimental section) and no obvious explanation can be offered, since vicinal action appears unlikely in this instance.

(11) For nomenclature of steroidal sapogenins, see Rosenkranz and Djerassi, *Nature*, **166**, 104 (1950).

to 22-isoallospirostan (desoxytigogenin, IVd). In all instances (IIa–IIg), the thioenol ethers were readily reconverted to the parent α,β-unsaturated ketone by acid hydrolysis.



The stability of the 3-benzylthioenol ether grouping toward lithium aluminum hydride[12] has already been employed for the reduction of a C-17 carbonyl group.[2] Application of the lithium aluminum hydride reduction to the thioenol ethers of progesterone (IIa) and 17α-hydroxyprogesterone (IIc) led predominantly to the 20β-hydroxy isomer VI and on hydrolysis to the α,β-unsaturated ketones VIIa and VIIb. Δ⁴-Pregnen-17α,20β-diol-3-one (VIIb) is new and while the corresponding 17α-desoxy analog VIIa has been described[13] the present method appears to be superior.



The reaction of benzyl mercaptan with 16-dehydroprogesterone (VIII), a substance with two α,β-unsaturated carbonyl functions in one molecule, represents an excellent example of how the reaction can be directed by proper choice of conditions. With pyridine hydrochloride as condensing agent, the 3-benzylthioenol ether IX could be isolated in 43% yield and its structure proved by desulfurization to Δ³,⁵,¹⁶-pregnatrien-20-one (XII) and subsequent reduction to allopregnan-20-one (IVa). The comparatively low yield was due to the fact that some addition to the Δ¹⁶-20-ketone system occurs even under those conditions as demonstrated by the formation of Δ⁵-16-thiobenzylpregnen-3β-ol-20-one 3-acetate (XIVa) from Δ⁵,¹⁶-pregnadien-3β-ol-20-one 3-acetate (XIII) with pyridine hydrochloride. In the presence of a basic catalyst (piperidine), 1,4-(or 3,4)-addition to the Δ¹⁶-20-ketone system of 16-dehydroprogesterone (VIII) (as well as of XIII) could be achieved with great facility. This is in striking contrast to the total lack of reactivity of the Δ⁴-3-keto system toward base-catalyzed 1,4-addition of benzyl mercaptan[14] and must be clearly due to steric hindrance. The ready addition of benzyl mercaptan finds a close parallel in the reaction of methoxide ion with Δ¹⁶ - 20 - ketosteroids[1,15] under mild conditions. Thus 16-dehydroprogesterone led in 76% yield to 16-thiobenzylprogesterone (XI) which could also be obtained by acid hydrolysis of its thioenol ether X, which in turn was isolated in the more drastic *p*-toluenesulfonic acid-catalyzed condensation of benzyl mercaptan with 16-dehydroprogesterone (VIII). As was to be expected, desulfurization of the 16-thiobenzyl 3-benzylthioenol ether (X) afforded Δ³,⁵-pregnadien-20-one (IIIa), identical with the specimen synthesized by hydrogenolysis of progesterone 3-benzylthioenol ether (IIa). It is thus clearly demonstrated that while thioenol ether formation proceeds only in the presence of acid catalysts, the 1,4-(or 3,4)-addition to a sterically unhindered α,β-unsaturated carbonyl system can occur with either acid or base.

Finally, the preparation and some reactions of steroid sulfoxides were also investigated. Hydrogen peroxide oxidation in alcohol or dioxane solution of a benzylthioenol ether (II) proceeded readily to yield a benzylsulfoxidoenol ether XVI, a type of compound which does not appear to have been described earlier. Similar oxidation of the 16-thiobenzyl-20-ketone XIVa led to the corresponding sulfoxide XIVb. Both the sulfoxidoketone XIVb and the sulfoxidoenol ethers XVI were readily desulfurized with Raney nickel[16] to yield the ketone XVa and diene III, respectively. It is interesting to note that in the case of the sulfoxidoenol ether (XVI) conversion of the sulfur to the higher oxidation state resulted in a hypsochromic shift of the characteristic ultraviolet maximum of II at 268 to 258 mμ and in a strikingly greater resistance toward acid hydrolysis. An increased positive charge on the sulfur atom in the

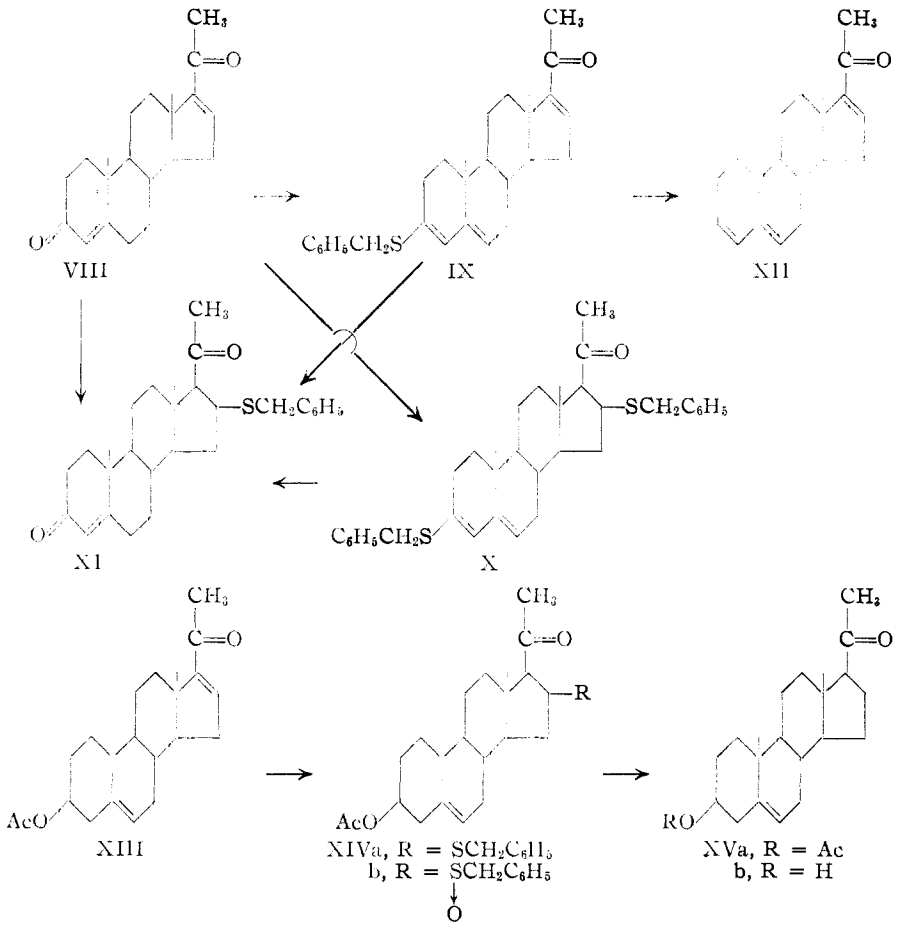
(12) The oxygen analogs (V) share this property [*cf.* Meystre, *et al.*, *Helv. Chim. Acta*, **32**, 1758, 1978 (1949), and Rosenkranz and Kaufman, U. S. Patent application 16,886].

(13) Wieland and Miescher, *Helv. Chim. Acta*, **32**, 1922 (1949).

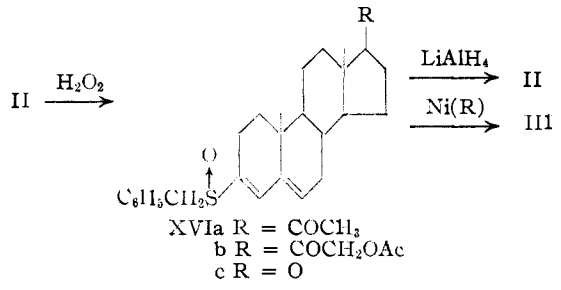
(14) Hauptmann (ref. 4) was unable to isolate any definite reaction products in the case of Δ⁴-cholesten-3-one (Ig). In our hands, progesterone (Ia), Δ⁴-androstene-3,17-dione (Ie) and testosterone (If) were recovered unchanged in the presence of piperidine, but considerable amounts of dibenzyl disulfide were formed.

(15) Fukushima and Gallagher, THIS JOURNAL, **72**, 2306 (1950); **73**, 196 (1951).

(16) The hydrogenolysis of sulfoxides is well known; *cf.* McOmie, *Ann. Repts. Prog. Chem.*, **45**, 200 (1949).



sulfoxide resulting in smaller electron release accounts for the resistance toward acid hydrolysis and the lowered u.v. maximum, since it would involve a higher energy transition state (adjacent positive charges).



The acid-*stable* sulfoxidoenol ethers (XVI) could be converted to the acid-*labile* thioenol ethers (II) as exemplified by the lithium aluminum hydride reduction[17] of Δ^4-androstene-3,17-dione 3-benzylsulfoxidoenol ether (XVIc) to testosterone 3-benzylthioenol ether (IIf). It is thus possible to protect a Δ^4-3-keto function under certain conditions in the presence of acidic reagents.

Neither 16-thiobenzylprogesterone (XI) nor progesterone 3-benzylsulfoxidoenol ether (XVIa) exhibited any progestational activity when tested in ovariectomized rabbits in ten times the threshold dose of progesterone.

(17) The reduction of a sulfoxide to a thioether by means of lithium aluminum hydride has been reported in general terms in the abstracts of a lecture given by W. G. Brown in the 1950 Spring Lecture Series of the North Jersey Section, A. C. S., New Brunswick, N. J. No specific example was recorded.

Experimental[18]

Δ^4-Androstene-3,17-dione 3-Benzylthioenol Ether (IIe).[2]—A solution of 3.0 g. of Δ^4-androstene-3,17-dione (Ie) in 150 cc. of benzene was dried by distilling 25 cc.; 0.1 g. of pyridine hydrochloride, 10 cc. of absolute ethanol and 3 g. of benzyl mercaptan were added and the mixture was refluxed for three hours. After dilution with ether, washing with carbonate solution, drying and evaporating, the residue was crystallized from methanol yielding 3.3 g. (81%) of colorless needles of the enol ether IIe with m.p. 175–178°, ultraviolet maximum at 268 mμ (log ε 4.39). No depression in m.p. was observed on admixture with a specimen prepared by the alternate condensation procedure.[2]

Testosterone 3-Benzylthioenol Ether (IIf).[2]—The reaction was carried out as above with 1.9 g. of testosterone (If), 1.5 g. of benzyl mercaptan, 0.1 g. of pyridine hydrochloride, 10 cc. of ethanol and 150 cc. of benzene; yield 1.5 g. (57%), m.p. 165–167.5°, $[\alpha]^{20}D$ −117° (dioxane), ultraviolet maximum at 268 mμ (log ε 4.38).

Δ^4-Cholesten-3-one 3-Benzylthioenol Ether (IIg).[2,9]—A 79% yield of thioenol ether with m.p. 120–121°, ultraviolet maximum at 268 mμ (log ε 4.20) was realized when 3.0 g. of Δ^4-cholesten-3-one (Ig) was condensed with 6.0 g. of benzyl mercaptan by the pyridine hydrochloride method. The same ratio of reactants in the presence of zinc chloride gives a nearly quantitative yield of mercaptole.[4]

Δ^4-22-Isospirosten-3-one[11] 3-Benzylthioenol Ether (IId).—Condensation of 8 g. of ketone Id with 8 g. of benzyl mercaptan in 150 cc. of benzene in the presence of 1.5 g. of pyridine hydrochloride and 25 cc. of ethanol gave 6.7 g. (67%) of enol ether IId as minute needles with m.p. 158–160°. Several recrystallizations from acetone raised the m.p. to 165–167°, $[\alpha]^{20}D$ −146.9° (dioxane), ultraviolet maximum at 268 mμ (log ε 4.31).

Anal. Calcd. for $C_{34}H_{46}O_2S$: C, 78.72; H, 8.93; S, 6.16. Found: C, 78.62; H, 8.85; S, 6.40.

Acid hydrolysis (one hour refluxing) regenerated Δ^4-22-isospirosten-3-one (Id).

$\Delta^{3,5}$-22-Isospirostadiene (IIId).[11]—The desulfurization of the above enol ether (5.0 g.) was carried out by refluxing with 800 cc. of acetone and 70 g. of W-2 Raney nickel catalyst[19] for one hour and then allowing the solution to stand at room temperature for 24 hours. Filtration, evaporation to dryness and recrystallization from acetone–methanol afforded 3.0 g. (78%) of the diene IIId with m.p. 167–168°, $[\alpha]^{20}D$ −150°, ultraviolet maxima at 228 mμ (log ε 4.27) and 234 mμ (log ε 4.30); reported[20] m.p. 168–169°.

(18) All melting points are corrected and were determined on the Kofler block. Unless noted otherwise, rotations were carried out in chloroform and ultraviolet absorption spectra in 95% ethanol solution. We are indebted to the Srtas. Paquita Revaque and Maria Eugenia Frontana for these measurements, to Srta. Amparo Barba of our Microanalytical Department for the C and H analyses, and to Mr. Joseph F. Alicino, Metuchen, N. J., for the S determinations. Sr. Humberto Estrada assisted in certain phases of the experimental work.

(19) Adkins and Pavlic, THIS JOURNAL, **69**, 3039 (1947).

(20) Marker and Turner, *ibid.*, **63**, 767 (1941).

Anal. Calcd. for $C_{27}H_{40}O_2$: C, 81.76; H, 10.16. Found: C, 81.60; H, 10.31.

22-Isoallospirostan (Desoxytigogenin) (IVd).[11]—Hydrogenation of 1.0 g. of the diene IIId in 100 cc. of ethyl acetate solution with 0.1 g. of 10% palladium-on-charcoal catalyst resulted in the uptake of 2 moles of hydrogen in 1.5 hours. Crystallization from methanol–ethyl acetate gave 0.82 g. of 22-isoallospirostan with m.p. 171–174°, tetranitromethane test negative. The analytical sample had m.p. 176–177.5°, $[\alpha]^{20}D$ −73.9°; reported[20] m.p. 173°.

Anal. Calcd. for $C_{27}H_{44}O_2$: C, 80.94; H, 11.07. Found: C, 81.08; H, 11.29.

Progesterone 3-Benzylthioenol Ether (IIa).—The condensation of progesterone (Ia) (8.0 g.) with benzyl mercaptan (5.0 g.) in the presence of pyridine hydrochloride (1.0 g.) was performed exactly as above (five hours refluxing) and produced 7.1 g. (65%) of enol ether IIa with m.p. 142–144°. When 0.2 g. of *p*-toluenesulfonic acid was substituted for the pyridine hydrochloride (ethanol was also omitted) the yield of enol ether IIa dropped to 34%. Recrystallization from acetone afforded the analytical sample with m.p. 146.5–148.5°, $[\alpha]^{20}D$ −50.7° (dioxane), ultraviolet maximum at 268 mμ (log ε 4.36).

Anal. Calcd. for $C_{28}H_{36}OS$: C, 79.96; H, 8.62; S, 7.62. Found: C, 79.89; H, 8.74; S, 7.50.

Refluxing of 0.2 g. of the thioenol ether IIa for one hour with 0.5% ethanolic hydrochloric acid led to 0.11 g. (73%) of progesterone (Ia), m.p. 127–129°, undepressed on admixture with the authentic hormone.

$\Delta^{3,5}$-Pregnadien-20-one (IIIa).—One and one-half grams of the thioenol ether IIa was desulfurized by refluxing with 200 cc. of acetone and 15 g. of W-2 Raney nickel[19] for four hours; yield 0.7 g. (69%), m.p. 139–142° (after recrystallization from acetone–methanol), $[\alpha]^{20}D$ −52.4°, ultraviolet maxima at 228 mμ (log ε 4.27) and 234 mμ (log ε 4.30).

Anal. Calcd. for $C_{21}H_{30}O$: C, 84.50; H, 10.13. Found: C, 84.35; H, 9.87.

The semicarbazone, prepared by the sodium acetate method, was recrystallized from methanol–chloroform and showed m.p. 248–250°, $[\alpha]^{20}D$ −31.1°, ultraviolet maximum at 232 mμ (log ε 4.49).

Anal. Calcd. for $C_{22}H_{33}ON_3$: C, 74.32; H, 9.35. Found: C, 74.18; H, 9.24.

The oxime exhibited m.p. 148–149°, $[\alpha]^{20}D$ −25.6° after recrystallization from methanol.

Anal. Calcd. for $C_{21}H_{31}ON$: C, 80.46; H, 9.96. Found: C, 80.78; H, 10.19.

Allopregnan-20-one (IVa).—The hydrogenation of the diene IIIa (palladium charcoal in ethyl acetate) proceeded in 85% yield to yield allopregnan-20-one with m.p. 136–137°, $[\alpha]^{20}D$ +100°, undepressed on admixture with a sample prepared from 22-isoallospirostan[11] (IVd)[21]; reported[22] m.p. 128–130° to 136–139°, $[\alpha]^{20}D$ +102°.

Lithium Aluminum Hydride Reduction of Progesterone 3-Benzylthioenol Ether (IIa).—A solution of 3.0 g. of the enol ether IIa in 300 cc. of ether was added over a period of ten minutes to a mixture of 0.3 g. of lithium aluminum hydride in 100 cc. of ether and then refluxed for 15 minutes. Decomposition with water (no acid added) followed by extraction with ether, washing and evaporation gave 1.82 g. (60%) of **Δ^4-pregnen-20β-ol-3-one 3-benzylthioenol ether (VIa)** from methanol with m.p. 138–140°, $[\alpha]^{20}D$ −128.4° (dioxane), ultraviolet maximum at 268 mμ (log ε 4.41).

Anal. Calcd. for $C_{28}H_{38}OS$: C, 79.57; H, 9.06; S, 7.57. Found: C, 79.46; H, 9.13; S, 7.71.

Δ^4-Pregnen-20β-ol-3-one (VIIa).—The above lithium aluminum hydride reduction product (1.0 g.) was hydrolyzed by refluxing for one-half hour with 90 cc. of methanol, 10 cc. of water and 0.5 cc. of hydrochloric acid. Dilution with water, followed by extraction with ether, evaporation and recrystallization from dilute methanol gave 0.4 g. (60%) of the unsaturated ketone VIIa with m.p. 169–171°, $[\alpha]^{20}D$ + 83°, ultraviolet maximum at 242 mμ (log ε 4.31); reported[13] m.p. 171–172°, $[\alpha]D$ + 84 ± 3°.

Anal. Calcd. for $C_{21}H_{32}O_2$: C, 79.70; H, 10.19. Found: C, 79.43; H, 10.09.

The acetate, after recrystallization from ether–pentane, exhibited m.p. 159–161°, $[\alpha]^{20}D$ +137°; reported[13] m.p. 159–159.5°, $[\alpha]D$ +140 ± 4°.

17α-Hydroxyprogesterone 3-Benzylthioenol Ether (IIc).—17α-Hydroxyprogesterone (Ic)[23] was converted in 60% yield into its thioenol ether IIc by the pyridine hydrochloride method illustrated above. The analytical sample crystallized as small needles from acetone–methanol with m.p. 182–183°, $[\alpha]^{20}D$ −101.7°, ultraviolet maximum at 268 mμ (log ε 4.38).

Anal. Calcd. for $C_{28}H_{36}O_2S$: C, 77.02; H, 8.31; S, 7.33. Found: C, 77.17; H, 8.31; S, 7.60.

Acid hydrolysis of 100 mg. of the thioenol ether IIc afforded 40 mg. of pure 17α-hydroxyprogesterone (Ic), m.p. 217–219°.

Lithium Aluminum Hydride Reduction of 17α-Hydroxyprogesterone 3-Benzylthioenol Ether (IIc).—The reduction of 1.0 g. of enol ether IIc in 300 cc. of ether was carried out with 0.2 g. of lithium aluminum hydride exactly as described for the progesterone analog IIa. Recrystallization from methanol–acetone gave colorless crystals (62% yield) of **Δ^4-pregnen-17α,20β-diol-3-one 3-benzylthioenol ether (VIb)** with m.p. 194–196°, $[\alpha]^{20}D$ −135.2° (dioxane), ultraviolet maximum at 268 mμ (log ε 4.40).

Anal. Calcd. for $C_{28}H_{38}O_2S$: C, 76.67; H, 8.73; S, 7.29. Found: C, 76.70; H, 8.91; S, 7.49.

Δ^4-Pregnene-17α,20β-diol-3-one (VIIb).—Acid hydrolysis of the enol ether VIb in the usual manner led in 60% yield to Δ^4-pregnene-17α,20β-diol-3-one (VIIb), which after recrystallization from acetone–hexane showed m.p. 201–204° (when inserted at 190°), $[\alpha]^{20}D$ +68.2°, ultraviolet maximum at 240 mμ (log ε 4.29).

Anal. Calcd. for $C_{21}H_{32}O_3$: C, 75.86; H, 9.70. Found: C, 75.49; H, 9.53.

Desoxycorticosterone Acetate 3-Benzylthioenol Ether (IIb).—The pyridine hydrochloride method applied to 3.0 g. of desoxycorticosterone acetate (Ib) and 2 g. of benzyl mercaptan (2.5 hours refluxing) produced 2.3 g. (60%) of needles with m.p. 156–158°. The analytical sample was obtained from methanol–acetone with m.p. 162–164°, $[\alpha]^{20}D$ −23.7° (dioxane), ultraviolet maximum at 268 mμ (log ε 4.30).

Anal. Calcd. for $C_{30}H_{38}O_3S$: C, 75.28; H, 8.00; S, 6.68. Found: C, 75.39; H, 8.29; S, 6.77.

$\Delta^{3,5}$-Pregnadien-21-ol-20-one 21-Acetate (IIIb)[10] **(a) By Dehydration of Δ^5-Pregnene-3β,21-diol-20-one 21-Acetate.**—A solution of 2.0 g. of Δ^5-pregnene-3β,21-diol-20-one 21-acetate was refluxed with 4.0 g. of phosphorus pentoxide[24] and 125 cc. of benzene for three hours. The product was isolated by dilution with ether, washing with water, evaporation and chromatography of the oily residue. Recrystallization from acetone afforded 0.6 g. (32%) of colorless needles with m.p. 153–155°, $[\alpha]^{20}D$ +46.5°[10] ultraviolet maximum at 232 mμ (log ε 4.30).

Anal. Calcd. for $C_{23}H_{32}O_3$: C, 77.48; H, 9.04. Found: C, 77.09; H, 8.93.

(b) By Desulfurization of Desoxycorticosterone Acetate 3-Benzylthioenol Ether (IIb).—Regardless of the reaction conditions, apparently some reduction of the diene system invariably occurred and the diene could not be isolated uncontaminated.[10] The best results were obtained on refluxing an acetone solution of 0.5 g. of the enol ether IIb with 4 g. of W-2 Raney nickel for two hours; yield 0.3 g. (78%), m.p. 166–168°, $[\alpha]^{20}D$ +70.6°, ultraviolet maxima at 228 mμ (log ε 4.01) and 234 mμ (log ε 4.03).

Anal. Found: C, 77.11; H, 9.22.

Allopregnan-21-ol-20-one 21-Acetate (IVb).—Hydrogenation of the above diene (procedure b) gave 83% of allopregnan-21-ol-20-one 21-acetate (IVb) with m.p. 202–203°, $[\alpha]^{20}D$ +101.8°. The tetranitromethane test was negative and the product gave no depression in m.p. when mixed with an authentic specimen[21] prepared from allopregnan-20-one.

(21) Mancera, Rosenkranz and Djerassi, *J. Org. Chem.*, **16**, February (1951).

(22) Marker and Lawson, THIS JOURNAL, **61**, 852 (1939); Meystre and Miescher, *Helv. Chim. Acta*, **28**, 1497 (1945).

(23) Rosenkranz, Pataki, Kaufmann, Berlin and Djerassi, THIS JOURNAL, **72**, 4081 (1950).

(24) *Cf.* Burrows, Cook, Roe and Warren, *Biochem. J.*, **31**, 950 (1937).

Anal. Calcd. for $C_{23}H_{36}O_3$: C, 76.62; H, 10.06. Found: C, 76.79; H, 10.29.

$\Delta^{4,16}$-Pregnadiene-3,20-dione 3-Benzylthioenol Ether (IX).—Three grams of 16-dehydroprogesterone (VIII) was refluxed for four hours with 2 g. of benzyl mercaptan, 0.2 g. of pyridine hydrochloride, 10 cc. of absolute ethanol and 150 cc. of benzene. The usual work-up led to 1.7 g. (43%) of thioenol ether IX with m.p. 171–173°, $[\alpha]^{20}D$ −99.2° (dioxane), ultraviolet maxima at 242 mμ (log ε 4.27) (due to Δ^{16}-20-keto grouping) and 268 mμ (log ε 4.36).

Anal. Calcd. for $C_{28}H_{34}OS$: C, 80.33; H, 8.18; S, 7.65. Found: C, 80.58; H, 8.35; S, 7.72.

$\Delta^{3,5,16}$-Pregnatrien-20-one (XII).—The desulfurization of the enol ether IX (0.8 g.) was accomplished by refluxing in acetone with 8.0 g. of W-2 Raney nickel for three hours. The triene (0.4 g., 70%) was recrystallized from methanol–acetone as white needles with m.p. 149–150°, $[\alpha]^{20}D$ −114.9°, ultraviolet maxima at 234 mμ (log ε 4.48) and 318 mμ (log ε 1.75). Shoppee and Prins[25] obtained presumably the same compound in a somewhat impure state (m.p. 142–143°, $[\alpha]^{13}D$ −106°) as a by-product in the pyrolysis of 3-acetoxy-17-benzoyloxy-Δ^5-pregnen-20-one.

Anal. Calcd. for $C_{21}H_{28}O$: C, 85.08; H, 9.51. Found: C, 85.31; H, 9.61.

The oxime, obtained by the pyridine method, was recrystallized from methanol–chloroform, m.p. 172–174°, $[\alpha]^{20}D$ −121.7°.

Anal. Calcd. for $C_{21}H_{29}ON$: C, 80.98; H, 9.38. Found: C, 81.14; H, 9.41.

Hydrogenation of the triene XII proceeded rapidly in ethyl acetate solution with palladium-on-charcoal catalyst with consumption of three moles of hydrogen and led in 78% yield to allopregnan-20-one (IVa) with m.p. 134–135°, $[\alpha]^{20}D$ +100°.

16-Thiobenzylprogesterone 3-Benzylthioenol Ether (X).—A solution of 8.0 g. of 16-dehydroprogesterone (VIII) in 250 cc. of benzene was refluxed for four hours with 5.5 g. of benzyl mercaptan and 0.3 g. of *p*-toluenesulfonic acid. The usual work-up followed by recrystallization afforded 7.1 g. (51%) of faintly yellowish crystals with m.p. 126–128°, $[\alpha]^{20}D$ −92.8° (dioxane), ultraviolet maximum at 268 mμ (log ε 4.37); note absence of maximum at 242 mμ as compared to IX.

Anal. Calcd. for $C_{35}H_{42}OS_2$: C, 77.43; H, 7.80; S, 11.81. Found: C, 77.03; H, 7.88; S, 12.08.

The semicarbazone (pyridine method) was obtained as a white, microcrystalline powder after recrystallization from methanol–chloroform; m.p. 147–149°, ultraviolet maximum at 268 mμ (log ε 4.37).

Anal. Calcd. for $C_{36}H_{45}ON_3S_2$: C, 72.06; H, 7.56; N, 7.00; S, 10.69. Found: C, 71.98; H, 7.66; N, 7.16; S, 10.44.

Raney nickel desulfurization of the 16-thiobenzyl 3-benzylthioenol ether X afforded 73% of $\Delta^{3,5}$-pregnadien-20-one (IIIa) (*vide supra*) with m.p. 139–141°, $[\alpha]^{20}D$ −53.8°.

16-Thiobenzylprogesterone (XI) (a) By Addition of Benzylmercaptan to $\Delta^{4,16}$-Pregnadiene-3,20-dione (VIII).—A solution of 3.0 g. of $\Delta^{4,16}$-pregnadiene-3,20-dione (VIII), 3.0 cc. of piperidine and 2.0 g. of benzyl mercaptan in 150 cc. of benzene was concentrated slightly by distillation to ensure complete dryness and then refluxed for three hours. After the usual work-up, 16-thiobenzylprogesterone (3.2 g., 76%) crystallized from hexane–acetone with m.p. 143–145°, $[\alpha]^{20}D$ +61.5° (dioxane), ultraviolet maximum at 240 mμ (log ε 4.29).

Anal. Calcd. for $C_{28}H_{36}O_2S$: C, 77.01; H, 8.31; S, 7.34. Found: C, 76.91; H, 8.44; S, 7.40.

The 3-semicarbazone (sodium acetate method) exhibited m.p. 202–204°, ultraviolet maximum at 268 mμ (log ε 4.68).

Anal. Calcd. for $C_{29}H_{39}O_2N_3S$: C, 70.56; H, 7.96; N, 8.50; S, 6.48. Found: C, 70.42; H, 7.97; N, 8.80; S, 6.35.

(b) **By Acid Hydrolysis of $\Delta^{4,16}$-Pregnadiene-3,20-dione 3-Benzylthioenol Ether (IX).**—One-half gram of the benzylthioenol ether IX was refluxed with 100 cc. of methanol and 0.5 cc. of concd. hydrochloric acid for three hours. After processing as usual, 0.4 g. of 16-thiobenzylprogesterone (XI) was isolated with m.p. 142–145°, undepressed on admixture with a specimen prepared according to (a); the rotation and spectrum also were identical. In order to prove that the course of this reaction involved initial cleavage to 16-dehydroprogesterone (VIII) followed by addition of the liberated benzyl mercaptan to the Δ^{16}-double bond,[26] 1.0 g. of 16-dehydroprogesterone (VIII) was refluxed for three hours with 0.6 cc. of benzyl mercaptan, 1 cc. of concd. hydrochloric acid and 150 cc. of methanol. The 16-thiobenzylprogesterone (1.05 g.) thus isolated (m.p. 142–144°) proved to be identical in all respects (rotation, spectrum) with the material synthesized according to (a).

(c) **By Acid Hydrolysis of 16-Thiobenzylprogesterone 3-Benzylthioenol Ether (X).**—Employing the conditions outlined in (b), the thioenol ether X was converted to 16-thiobenzylprogesterone in nearly 90% yield.

Δ^5-16-Thiobenzylpregnen-3β-ol-20-one 3-Acetate (XIVa).—A solution of 2.0 g. of $\Delta^{5,16}$-pregnadien-3β-ol-20-one 3-acetate (XIII) in 70 cc. of benzene was allowed to stand at room temperature for four days with 3.0 g. of benzyl mercaptan and 4.0 cc. of piperidine. After washing with dilute hydrochloric acid, carbonate solution and water, evaporation and recrystallization from hexane, 2.05 g. (74%) of colorless crystals was obtained with m.p. 124–125°, $[\alpha]^{20}D$ −40.2° (dioxane), *no* ultraviolet maximum at 240 mμ.

Anal. Calcd. for $C_{30}H_{40}O_3S$: C, 74.96; H, 8.38; S, 6.65. Found: C, 74.83; H, 8.41; S, 6.89.

A polymorphic form of XIVa, m.p. 78–80°, $[\alpha]^{20}D$ −39.8° (dioxane), was isolated in 71% yield when the reaction was carried out by the pyridine hydrochloride method (five hours refluxing). Hydrogenolysis and peroxide oxidation gave the same products (*vide infra*) as the 125° form.

Desulfurization of either product (m.p. 80° or 125°) in the customary manner led in 90% yield to Δ^5-pregnen-3β-ol-20-one 3-acetate (XVa), m.p. 147–148°, $[\alpha]^{20}D$ +16°, and on saponification to the free pregnenolone (XVb), m.p. 188–190°, $[\alpha]^{20}D$ +25°; no depression in m.p. was observed on admixture with authentic specimens.

Δ^5-16-Sulfoxidobenzylpregnen-3β-ol-20-one 3-Acetate (XIVb).—A mixture of 2.5 g. of 16-thiobenzylpregnenolone acetate (XIVa), 150 cc. of ethanol, 5 cc. of saturated sodium carbonate solution and 15 cc. of 30% hydrogen peroxide was heated for five minutes on the steam-bath and worked up as usual; recrystallization from hexane–acetone gave 1.5 g. (58%) of sulfoxide XIVb with m.p. 141–143.5°, $[\alpha]^{20}D$ −35.3° (dioxane).

Anal. Calcd. for $C_{30}H_{40}O_4S$: C, 72.55; H, 8.11; S, 6.44. Found: C, 72.30; H, 8.22; S, 6.51.

Desulfurization with W-2 Raney nickel gave 75% of Δ^5-pregnen-3β-ol-20-one 3-acetate (XVa).

Δ^4-Androstene-3,17-dione 3-Benzylsulfoxidoenol Ether (XVIc).—A hot solution of 1.0 g. of the thioenol ether IIe in 150 cc. of dioxane was treated with 10 cc. of 30% hydrogen peroxide solution for five minutes on the steam-bath and then poured into water. Extraction with ether, evaporation and recrystallization of the solid residue from a mixture of methanol and methylene chloride gave 0.7 g. (67%) of the corresponding sulfoxidoenol ether XVIc with m.p. 217–219° (dec.), $[\alpha]^{20}D$ −113° (dioxane), ultraviolet maximum at 258 mμ (log ε 4.35).

Anal. Calcd. for $C_{26}H_{32}O_2S$: C, 76.43; H, 7.89; S, 7.83. Found: C, 76.60; H, 7.87; S, 7.56.

Acid hydrolysis in the manner given above for the cleavage of thioenol ethers but refluxing for two hours resulted in a 63% recovery of sulfoxide. Hydrogenolysis afforded 58% of $\Delta^{3,5}$-androstadien-17-one (IIIe) with m.p. 80–82°, ultraviolet maxima at 228 mμ (log ε 4.29) and 234 mμ (log ε 4.31).

Lithium Aluminum Hydride Reduction of Δ^4-Androstene-3,17-dione 3-Benzylsulfoxidoenol Ether (XVIc).—A solution of 2.0 g. of the sulfoxide XVIc in 350 cc. of dry tetrahydrofuran was added over a period of 15 minutes to a solution of 0.8 g. of lithium aluminum hydride in 50 cc. of tetrahydrofuran and the mixture was then refluxed for an additional 15 minutes. After addition of water, the product was isolated by extraction with ether, washing with water, drying and evaporating. Crystallization from methanol afforded 1.1 g. (57%) of testosterone 3-benzylthioenol

(25) Shoppee and Prins, *Helv. Chim. Acta*, **26**, 1004 (1943).

(26) Ralls, Dodson and Riegel (ref. 4a) reported a 1,6-addition of ethyl mercaptan to $\Delta^{3,5}$-cholestadien-7-one in the presence of hydrogen chloride.

ether (IIf) with m.p. 164–166°, $[\alpha]_D^{20}$ −114° (dioxane), ultraviolet maximum at 268 mμ (log ε 4.31). Acid hydrolysis yielded 82% of testosterone (If) with m.p. 152–154°, $[\alpha]_D^{20}$ +109.7°, ultraviolet maximum at 242 mμ (log ε 4.25).

Progesterone 3-Benzylsulfoxidoenol Ether (XVIa).—The oxidation of the thioenol ether IIa (3.00 g.) was carried out on the steam-bath by heating for 15 minutes with 350 cc. of dioxane, 20 cc. of 30% hydrogen peroxide and 5 cc. of sodium carbonate solution. After dilution with water, the sulfoxide was extracted with a mixture of methylene chloride and ether and recrystallized from methylene chloride–methanol; yield 1.7 g. (48%), m.p. 200–201°, $[\alpha]_D^{20}$ −94.3° (dioxane), ultraviolet maximum at 258 mμ (log ε 4.32).

Anal. Calcd. for $C_{28}H_{36}O_2S$: C, 77.02; H, 8.31; S, 7.33. Found: C, 77.45; H, 8.51; S, 7.30.

Desoxycorticosterone Acetate 3-Benzylsulfoxidoenol Ether (XVIb).—A solution of 5.0 g. of desoxycorticosterone acetate 3-benzylthioenol ether (IIb) in 300 cc. of dioxane was allowed to stand at room temperature for 48 hours with 20 cc. of 30% hydrogen peroxide. The usual work-up followed by recrystallization from acetone–methanol produced 2.6 g. (50%) of desoxycorticosterone acetate 3-benzylsulfoxidoenol ether (XVIb) with m.p. 171–173° (dec.), $[\alpha]_D^{20}$ −82.8° (dioxane), ultraviolet maximum at 258 mμ (log ε 4.32).

Anal. Calcd. for $C_{30}H_{38}O_4S$: C, 72.84; H, 7.74; S, 6.46. Found: C, 72.89; H, 8.10; S, 6.40.

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The Preparation of Desoxynucleotides[1]

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Desoxynucleotides have been isolated in good yield from enzyme digests of DNA by the method of ion-exchange chromatography. The techniques of enzymatic digestion, ion-exchange separation of the mononucleotides and the preparation of these as solid products are described. The mononucleotides have been analyzed spectrophotometrically and with respect to nitrogen and phosphorus composition; some preliminary biological characterizations of these products have been carried out.

Introduction

Ever since the double enzymatic hydrolysis procedure of Klein[1a] offered the possibility of preparing quantities of mixed nucleotides from desoxyribonucleic acid (DNA) with comparative ease, the problem of obtaining the individual compounds in pure form has been one of separation from the mixtures obtained. In view of the speed, precision, yield and mild conditions which characterize the ion-exchange separation of the analogous ribonucleotide hydrolysis mixtures,[2] the adaptation of this method to the resolution of the enzymatically produced mixture was sought as a means of making the desoxy compounds readily available for chemical and biological study. In this communication, such a combined procedure is described, together with preliminary characterizations of the nucleotides thus prepared.

Experimental

Material.—Polymerized DNA was prepared from calf thymus by the method of Mirsky and Pollister.[3] Desoxyribonuclease was prepared from calf pancreas by McCarty's procedure[4] and stored in the frozen-dried state. The activity of the enzyme was comparable to that prepared by McCarty. Alkaline intestinal phosphatase, prepared from calf intestinal mucosa according to Schmidt and Thannhauser[5] without purification by alumina or kaolin adsorption, was obtained from the Armour Laboratories. The dried material contained fifteen phosphatase units per mg. Although this preparation was highly contaminated with adenosine deaminase, the latter enzyme did not interfere with the procedure, since it specifically attacks the nucleoside.[6]

Enzymatic Digestion.[7]—To 10 g. of thymus DNA, dissolved in 2 l. of water, was added 70 cc. of 0.1 *M* magnesium chloride. The solution was adjusted to *p*H 7.2 and 25 mg. of desoxyribonuclease, dissolved in water and previously adjusted to the same *p*H, was added to the DNA solution with vigorous stirring. As the reaction progressed, the solution was maintained at *p*H 7.2 by the addition of 0.5 *N* sodium hydroxide from a buret. The reaction was allowed to proceed to completion and required 13 cc. of 0.5 *N* sodium hydroxide. Since with this amount of enzyme the hydrolysis takes between seven and eight hours, the digest is generally placed overnight in the cold before subsequent phosphatase hydrolysis.

The digest is then made approximately 0.005 *M* with respect to sodium arsenate by adding 20 cc. of 0.5 *M* sodium arsenate and is then adjusted to *p*H 8.4. Four grams of intestinal phosphatase, dissolved in 50 cc. of water and adjusted to the same *p*H, was added to the nuclease-treated DNA and the reaction followed by titration with 0.5 *N* sodium hydroxide as above. This hydrolysis procedure requires 45 cc. of 0.5 *N* sodium hydroxide and takes nine to ten hours for completion.

The reaction is stopped, and at the same time the digest is made ready for ion-exchange separation, by the addition of concentrated ammonia to a final concentration of 1 *M* ammonium hydroxide.

Ion-Exchange Separation.—The enzymatic digest of 10 g. of DNA in about 2 l. of solution at *p*H *ca.* 10 is absorbed on a strong-base chloride-form fine-mesh ion-exchange column 33 sq. cm. × 6.0 cm. in height. This column is then washed with the following: (1) water, to remove ammonia; (2) 0.01 *M* ammonium chloride (12–15 l.), until the *p*H of the effluent falls to 7, to remove bases and nucleosides; (3) 0.01 *M* hydrochloric acid (15–20 l.) to remove all nucleotides. Polynucleotides and undigested nucleic acid are left on the column; this is the principal purpose of the preliminary step, which may be omitted if desired.

The 0.01 *M* hydrochloric acid solution is made alkaline with ammonium hydroxide and adsorbed on the separation column, 33 sq. cm. × 12 cm. of the same material. The following reagents are then used in succession: (1) 0.01 *M* ammonium chloride until the *p*H falls to 6; (2) 0.001 *M* hydrochloric acid to remove desoxycytidylic acid (and 5-methylcytidylic acid)[8]; (3) 0.002 *M* hydrochloric acid to remove desoxyadenylic acid; (4) 0.003 *M* hydrochloric acid to remove thymidylic acid; (5) 0.005 *M* hydrochloric acid

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