there was obtained an oil which solidified mostly. Fractional recrystallization from ethanol afforded 0.01 g. of the starting material (XII), m.p. 225–235°; the pure sample, m.p. and mixed m.p. 247–249°. On standing for a few days, the mother liquors of XII deposited 0.02 g. (50%) of γ-tetrahydrosantonin as colorless prisms, m.p. 88–94°; the pure sample, m.p. and mixed m.p. 98–100° (from dilute ethanol).

The *trans*-ketol acetate (0.05 g.), as described above, was refluxed 24 hr. with zinc dust to give an oil which solidified mostly. Trituration with a little methanol afforded 0.025 g. (62%) of γ-tetrahydrosantonin (I), m.p. 83–90°; the pure sample, m.p. and mixed m.p. 98–100° (from methanol).

Clemmensen reduction of cis- and trans-2-acetoxy-γ-tetrahydrosantonin (XII *and* XIII). Employing the conditions described earlier for 2-acetoxy-α-tetrahydrosantonin,[1] the *cis*-ketol acetate (XII, 0.10 g.) was reduced by the Martin modification of the Clemmensen method. There was obtained 0.07 g. (92%) of the 3-desoxy-γ-tetrahydrosantonin (V), m.p. 73–81°; the pure sample, m.p. and mixed m.p. 85–86° (from ethanol).

On a similar treatment, the *trans*-ketol acetate (XIII, 0.10 g.) gave 0.07 g. (92%) of the 3-desoxy compound (V), m.p. 69–76°; the pure sample, m.p. and mixed m.p. 85–86° (from ethanol).

Dimethylene thioketals of cis- and trans-2-acetoxy-γ-tetrahydrosantonin (XII *and* XIII). As described earlier for the 2-acetoxy-α-tetrahydrosantonin,[1] 0.34 g. of the *cis*-ketol acetate (XII) was allowed to stand 48 hr. with 0.3 cc. of ethane dithiol and 0.7 cc. of boron trifluorid-ether complex in 5 cc. of acetic acid. The crude dimethylene thioketal (XVII, quantitative), melting in the range 140–161°, was recrystallized from ethanol to give 0.22 g. (57%) of colorless prisms, m.p. 176–177.5°; $[\alpha]_D^{26}$ −58.3° ($CHCl_3$; *c* 1.27); $\nu_{C=O}$ 1783 (γ-lactone) and 1751 cm.$^{-1}$ (acetyl) (in $CHCl_3$ solution).

Anal. Calcd. for $C_{19}H_{28}O_4S_2$: C, 59.36; H, 7.28. Found: C, 59.61; H, 7.12.

By the same procedure, the *trans*-ketol acetate (XIII, 0.50 g.) formed the dimethylene thioketal (XVIII, 0.59 g., 95%), melting in the range 130–152°. Recrystallization from ethanol gave 0.46 g. (74%) of colorless prisms, m.p. 163–165.5°; $[\alpha]_D^{26}$ −32.3° ($CHCl_3$; *c* 1.33); $\nu_{C=O}$ 1779 (γ-lactone) and 1748 cm.$^{-1}$ (acetyl) (in $CHCl_3$ solution).

Anal. Calcd. for $C_{19}H_{28}O_4S_2$: C, 59.36; H, 7.28. Found: C, 59.25; H, 7.57.

Attempted epimerization of the dimethylene thioketal (XVIII) *of* XIII. Exactly as described earlier for the *cis*-acetoxy thioketal in the α- series,[1] the dimethylene thioketal (XVIII) was heated to reflux in dioxane, but the starting material was completely recovered. When the refluxing time was prolonged to 50 hr., XVIII was converted to an oil which could not be induced to crystallize.

Reduction of the dimethylene thioketals (XVII *and* XVIII) *of the ketol acetates with Raney nickel.* As described earlier for the same derivatives of the ketol acetate in the α-series,[1] 0.06 g. of the dimethylene thioketal (XVII) of the *cis*-ketol acetate was heated to reflux 30 hr. with Raney nickel (0.6 g.) in dioxane (10 cc.). There was obtained 0.03 g. (82%) of the above 3-desoxy-γ-tetrahydrosantonin (V), m.p. 63–72°; the pure sample, m.p. and mixed m.p. 85–86°.

By the same procedure, the dimethylene thioketal (XVII) of the *trans*-ketol acetate was converted to the 3-desoxy compound (V) in good yield.

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[CONTRIBUTION FROM THE RESEARCH LABORATORIES OF SYNTEX S.A.]

Steroids. CXIX.[1] The Preparation of Some Vicinal Glycols in the Cortical Hormone Series

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Osmylation of Δ^6-dehydrocortisone acetate and Δ^6-dehydro-9α-fluorohydrocortisone acetate proceeds in dioxane solution to yield the corresponding 6α,7α-dihydroxy analogs of cortisone acetate and 9α-fluorohydrocortisone acetate. When Δ^6-dehydroprednisone acetate is similarly treated a mixture of 6α,7α-dihydroxyprednisone acetate and 1α,2α-dihydroxy-Δ^6-dehydrocortisone acetate is obtained.

The findings that in the cortical hormone series 16α-hydroxylation[2] as well as 16α,17α-acetal or ketal formation[3] markedly influence biological properties prompted us to investigate a number of steroid vicinal glycols. This paper reports the synthesis of four such compounds.

Thus when Δ^6-dehydrocortisone acetate (Ia)[4] was allowed to stand for four or five days at room temperature in dioxane solution with osmium tetroxide there was obtained, following hydrogen sulfide decomposition of the osmic ester,[5] a mixture of starting material and 6α,7α-dihydroxycortisone acetate (IIa). This mixture was readily resolved by chromatography to provide the pure 6α,7α-dihydroxy compound (IIa), characterized as its 6α,7α-acetonide (III). In our hands the addition of small quantities of pyridine[2] to the osmylation mixture did not improve the yield.

An identical procedure with Δ^6-dehydro-9α-

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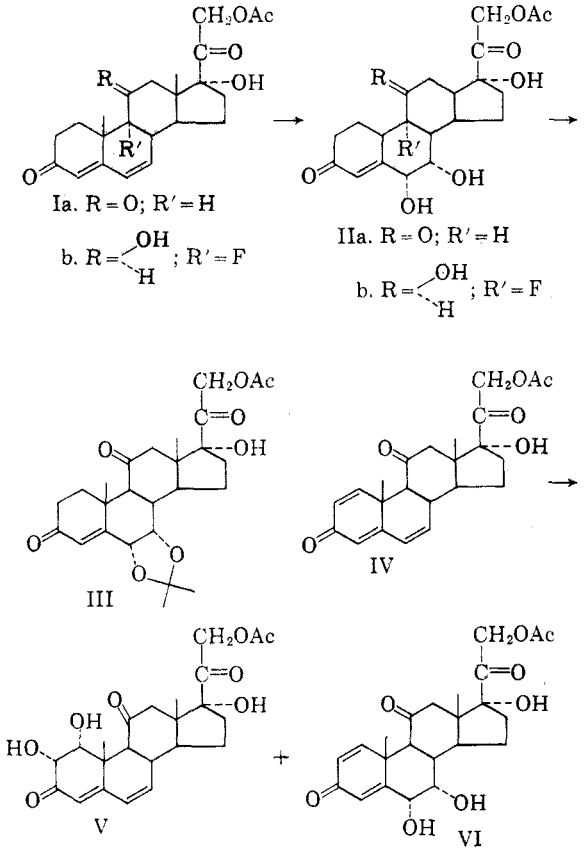
fluorohydrocortisone acetate (Ib)[6] led to the isolation of 6α,7α-dihydroxy-9α-fluorohydrocortisone acetate (IIb).

When Δ⁶-dehydroprednisone acetate (IV)[7] was treated under the above conditions a difficultly separable mixture of 1α,2α-dihydroxyΔ⁶-dehydrocortisone acetate (V) and 6α,7α-dihydroxyprednisone acetate (VI) resulted. While it was not possible to obtain satisfactory analytical results for the 1α,2α-dihydroxy compound (V), the spectral data proved to be in accord with the proposed structure.



The assignment of the α- configurations to the above products has been made solely on the basis that attack from the rear[8] is a more probable steric course considering the large bulk of osmium tetroxide.

Bioassays[9] of IIa, IIb, III, and VI indicate that all the compounds are considerably less potent than their parent steroids in terms of thymolytic and anti-inflammatory activity.

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(9) We wish to thank Dr. R. I. Dorfman of the Worcester Foundation, Shrewsbury, Mass., for these assays.

EXPERIMENTAL[10]

6α,7α-Dihydroxycortisone acetate (IIa). Dioxane (50 ml.) containing 3.46 g. of Δ⁶-dehydrocortisone acetate (Ia)[4] and 3.46 ml. of pyridine was allowed to stand at room temperature for 5.5 days with 2.0 g. of osmium tetroxide. The mixture was then saturated with hydrogen sulfide and filtered through a pad of filter aid. The resultant colored filtrate was evaporated to dryness and taken up in 50 ml. of methanol. By stirring for 20 min. with 10 g. of neutral alumina and 2 g. of decolorizing carbon and then filtering, the solution was almost completely decolorized and gave upon evaporation to dryness 2.6 g. of noncrystalline material, λ_{max}^{EtOH} 238–240 and 280 mμ, log ε 3.84 and 4.00. Following adsorption on 50 g. of Florisil and elution with methylene chloride, 0.7 g. of crude starting material was recovered as indicated by its ultraviolet spectrum (λ_{max}^{EtOH} 280–282 mμ, log ε 4.21). Further elution with increasing polar mixtures of methylene chloride–acetone provided in the methylene chloride–acetone (1:1) fraction 1.06 g., m.p. 225–240°, λ_{max}^{EtOH} 236–238 mμ, log ε 3.81. By repeated recrystallizations from methanol–ethyl acetate 0.17 g. of pure 6α,7α-dihydroxycortisone acetate (IIa) was obtained, m.p. 273–276°, $[\alpha]_D$ +210° (pyridine), λ_{max}^{EtOH} 238–240 mμ, log ε 4.00.

Anal. Calcd. for $C_{23}H_{30}O_8$: C, 63.58; H, 6.96; O, 29.46. Found: C, 64.05; H, 7.18; O, 28.98.

6α,7α-Dihydroxycortisone acetate 6,7-acetonide (III). To 20 ml. of acetone containing 150 mg. of 6α,7α-dihydroxycortisone acetate (IIa) was added 5 drops of 78% perchloric acid. After 1 hr. at room temperature 5 drops of pyridine was added and the resultant solution was evaxporated to dryness under reduced pressure. Water (5 ml.) was added to the residue and it was then extracted several times with 10 ml. of ethyl acetate. The pooled extracts were washed to neutrality with water, dried over sodium sulfate, and evaporated to dryness. The residue upon trituration with methanol gave 60 mg. of crystals m.p. 273–277°. Four additional recrystallizations from the same solvent furnished the pure acetonide III m.p. 279–282°, $[\alpha]_D$ +242° (chloroform) λ_{max}^{EtOH} 240 mμ, log ε 4.11.

Anal. Calcd. for $C_{26}H_{34}O_8$: C, 65.80; H, 7.22; O, 26.98. Found: C, 66.05; H, 7.15; O, 26.76.

6α,7α-Dihydroxy-9α-fluorohydrocortisone acetate (IIb). Following the procedure previously described, 1.0 g. of Δ⁶-dehydro-9α-fluorohydrocortisone acetate (Ib)[6] was treated for 5 days with a large excess of osmium tetroxide (1.0 g.). The resulting semicrystalline product, 1.0 g., λ_{max}^{EtOH} 238–240 mμ, log ε 3.92, was then chromatographed on 20 g. of silica gel. Elution with benzene–ether (2:3) and pure ether yielded 0.58 g. of crystals m.p. 223–227° which after repeated recrystallization from acetone–ether exhibited the m.p. 251–253°, $[\alpha]_D$ +68° (pyridine), λ_{max}^{EtOH} 238 mμ, log ε 3.95.

Anal. Calcd. for $C_{23}H_{31}O_8F$: C, 60.78; H, 6.87. Found: C, 61.23; H, 6.96.

1α,2α-Dihydroxy-Δ⁶-dehydrocortisone acetate (V) *and 6α,7α-dihydroxyprednisone acetate* (VI). To 100 ml. of dioxane containing 1.65 g. of Δ⁶-dehydroprednisone acetate (IV)[7] was added 1.0 g. of osmium tetroxide and the mixture was kept at room temperature for 4 days. Following saturation with hydrogen sulfide and filtration through a pad of filter aid there was obtained a clear solution which upon evaporation to dryness provided 1.40 g. of crystalline material. This was then adsorbed on 60 g. of Florisil whereupon elution with methylene chloride–acetone (8:2) and (1:1) provided 1.10 g. of crystals m.p. 239–241°, λ_{max}^{EtOH} 244 mμ and 276–280 mμ, log ε 3.94 and 4.06. After six recrystallizations from methanol 85 mg. of pure 6α,7α-dihydroxyprednisone acetate (VI) was obtained m.p. 290–292° dec., $[\alpha]_D$ +109° (pyridine), λ_{max}^{EtOH} 238 mμ, log ε 4.11.

(10) We are indebted to Dr. L. Throop and staff for all spectral and rotational determinations.

Anal. Calcd. for $C_{23}H_{28}O_8$: C, 63.88; H, 6.53; O, 29.60. Found: C, 63.78; H, 6.81; O, 29.36.

Evaporation of the mother liquors from the first recrystallization yielded 0.5 g. of noncrystalline material λ_{max}^{EtOH} 234–238 mμ, log ε 3.88 whereas combination and evaporation of the mother liquors from the following two recrystallizations gave *ca.* 0.2 g. of crystals λ_{max}^{EtOH} 276–280 mμ, log ε 3.96. This latter substance was then adsorbed on 6 g. of silica gel from a solution of methylene chloride. Elution of the column with methylene chloride–acetone (9:1) led to 60 mg. of crystals which were recrystallized four times from acetone thus providing 1α,2α-dihydroxy-Δ^6-dehydrocortisone acetate (V) m.p. 235–238°, $[\alpha]_D$ +230° (pyridine), λ_{max}^{EtOH} 280 mμ, log ε 4.32. The poor analytical results cannot be ascribed to dehydration since the substance gave no color with ferric chloride and the ultraviolet absorption spectra was not altered by addition of alkali.

Anal. Calcd. for $C_{23}H_{28}O_8$: C, 63.88; H, 6.53. Found: C, 64.92; H, 6.83.

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Alkaloids of Tobacco Smoke. I. Fractionation of Some Tobacco Alkaloids and of the Alkaloid Extract of Burley Cigarette Smoke by Gas Chromatography[1]

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An alkaloid extract from Burley tobacco cigarette smoke was separated by gas chromatography on polyglycol columns. It was necessary to perform the separation under three sets of conditions to overcome difficulties associated with the wide boiling range of the mixture and the relatively massive amount of nicotine present. There appears to be a minimum of sixteen alkaloidal or basic compounds, in addition to nicotine, boiling above 150–170° in the extract. Besides its analytical features, the gas chromatographic method is valuable in isolation and purification of the alkaloids.

It has been known for many years that other alkaloids in small quantities accompany nicotine in tobacco smoke,[2] but much uncertainty exists as to the identity and amounts of these compounds. The recent paper chromatographic work of Kuffner, Schick, and Bühn[3] has done much to clarify in a qualitative manner the alkaloidal content of cigar smoke. They were able to show that many of the alkaloids in the smoke were present in the tobacco itself. However, definitive studies are lacking on cigarette smoke, which differs from cigar smoke in several respects. We have recently undertaken a study of the alkaloids in a continuation of our work on the chemical composition of cigarette smoke.[4]

It was anticipated that the tobacco alkaloids, generally boiling in the range 200–300°, might be subject to separation by the versatile technique of gas chromatography. This hope was realized and in a preliminary communication[5] we reported the successful application of gas chromatography to these compounds. It was found that good separation of a majority of the alkaloids studied could be achieved at moderate temperatures (about 190°) on 1 meter columns containing certain polyglycols as the stationary liquid phase. The list of known alkaloids studied has been extended since this initial report; a complete list with the retention times on three different columns is provided as Table I.

TABLE I

GAS CHROMATOGRAPHY OF INDIVIDUAL TOBACCO ALKALOIDS

Liquid Phase	Columns and Conditions: Polypropylene glycol[a]	Polybutylene glycol[b]	Polyethylene glycol[c]
Temp., °C.	190	180	190
He flow, ml./min.	45	50	48
	Retention Time, Min.		
3-Pyridyl methyl ketone	4.3	3.1	4.3
3-Pyridyl ethyl ketone	6.1	5.0	5.3
3-Pyridyl *n*-propyl ketone	8.1	7.0	6.6
Nicotine	8.6	8.2	5.2
Nornicotine	16.1	14.3	12.3
Myosmine	16.4	14.7	13.4
Anabasine	19.4	18.1	13.8
Nicotyrine	21.0	18.3	19.4
Metanicotine	23.5	20.9	16.5
Anatabine	25.2	22.5	21.1
2,3′-Dipyridyl	31	26	29
N-Methyl nicotinamide	42	30	64
Nornicotyrine	73	55	101
Cotinine	79	63	85

[a] Mol. wt. 1025. [b] Mol. wt. 1500. [c] Mol. wt. 20,000.

(1) Supported by a grant from the Damon Runyon Memorial Fund. Presented at the Twelfth Tobacco Chemists' Research Conference, October 23, 1958, Durham, N. C.

(2) The literature has been recently reviewed: A. I. Kosak in *The Biologic Effects of Tobacco*, ed. by E. L. Wynder, Little, Brown and Co., Boston, Mass., 1955, p. 15; A. I. Kosak, *Experientia*, **10**, 69 (1954); L. Marion in *The Alkaloids*, ed. by R. H. F. Manske and H. L. Holmes, Acedemic Press, N. Y., N. Y., 1950, Vol. 1, p. 228.

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