last coupling reaction, the resin was washed thoroughly with CH_2Cl_2, transferred to a Büchner funnel with ethanol, and dried thoroughly *in vacuo*.

Cleavage and Purification of the Nonapeptide.—A 3.0-g portion of the protected nonapeptide resin was suspended in 15 ml of trifluoroacetic acid, and anhydrous HBr was bubbled slowly through the suspension for 90 min. The resin was filtered and washed three times with trifluoroacetic acid. The combined filtrates were evaporated under reduced pressure below 30°, and trifluoroacetic acid was again added and evaporated several times to remove excess HBr. The product was then dissolved in acetic acid and lyophilized to yield 735 mg of crude peptide. Hydrolysis and amino acid analysis of the crude nonapeptide gave approximately the expected amino acid ratios.

The crude nonapeptide (700 mg) was purified by a 99-transfer countercurrent distribution in 1-butanol–water–formic acid (50:50:1 by vol.). The principal component (k = 1.22) was isolated by combination and evaporation of the contents of tubes 40–70 and was subjected to a further 99-tube distribution in the system 1-butanol–pyridine–acetic acid–water (150:2:0.2:150 by vol.). The principal component (k = 0.11) was isolated by combination and evaporation of the contents of tubes 3–20. An aqueous solution of the product was lyophilized to yield 331 mg of a white solid.

The glycylasparylserylglycylglycylprolylleucylvalyl-S-benzylcysteine was shown to be homogeneous by paper chromatography and paper electrophoresis. The peptide had R_f 0.54 in 2-propanol–water (2:1 by vol.), R_f 0.84 in liquid phenol–water (3:1), and R_f 0.58 in 1-butanol–acetic acid–water (4:1:5). In paper electrophoresis (0.6 *M* pyridine–acetic acid, pH 6.5) the peptide moved as an anion with R_{Asp} 0.28. Hydrolysis and amino acid analysis gave the ratios Gly 3.4, Ser 1.0, Val 1.0, Pro 1.0, Leu 1.1, Asp 1.1, Cys + S-Bzyl Cys 0.94. The nonapeptide exhibited no catalytic activity for the hydrolysis of acetyl-L-phenylalanine ethyl ester when tested by the method of Woolley[8] at 0.15 mg/ml at any pH between 8.5 and 5.0.

(8) D. W. Woolley, *J. Am. Chem. Soc.*, **88**, 2330 (1966).

The Synthesis of the 2α-Methylthio Derivatives of Cortisone, Hydrocortisone, and Progesterone. The Reaction of Methanesulfenyl Chloride with Alkoxalylated Steroid Ketones

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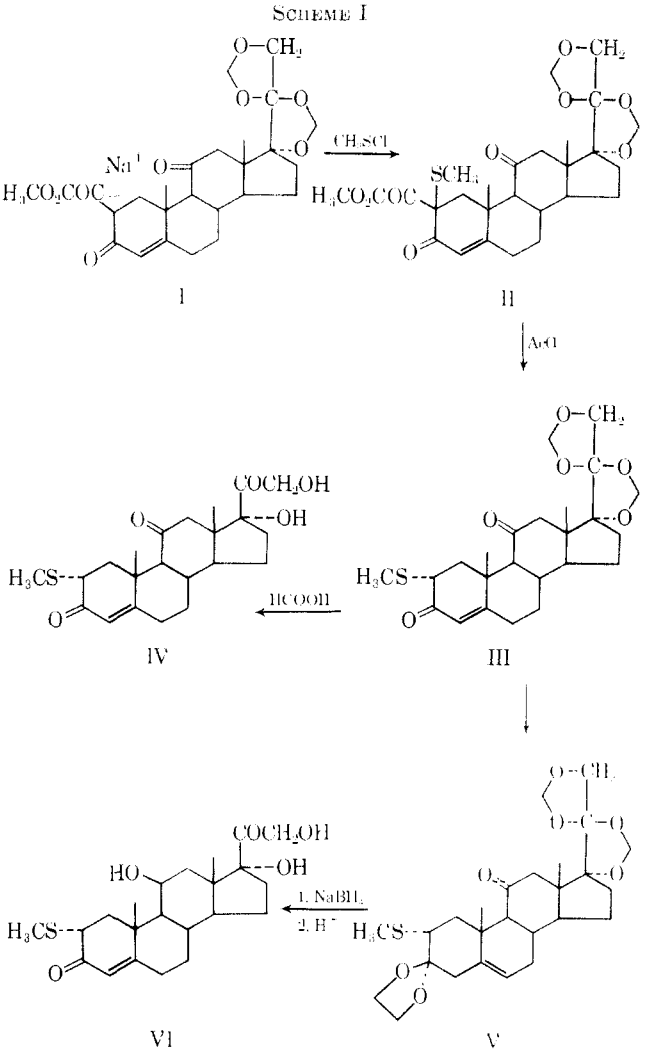
In continuation of our program in the steroid hormone analog field we wish to report the preparation of the 2α-methylthio derivatives of cortisone, hydrocortisone, and progesterone.[1] Introduction of the methylthio group was accomplished by the reaction of appropriate alkoxalylated steroids with methanesulfenyl chloride[7] and this reaction represents a further demonstration of the synthetic versatility of these steroid derivatives. The utility of alkoxalylated steroid ketones and/or the related hydroxymethylene ketones for the introduction of alkyl, aryl,[8] cyano, benzoyloxy, and hydroxyimino[9] groups as well as for the halogens, including fluorine, has been demonstrated.[10]

2α-Methylthiocortisone was prepared by the sequence I → IV (Scheme I). Reaction of the sodium

SCHEME I



salt (I)[11] of the bismethylenedioxy derivative[12] of 2-methoxalylcortisone with methanesulfenyl chloride gave the 2-methylthio-2-methoxalyl derivative II in 75% crude yield. Acetate-induced demethoxalylation afforded 57% of the 2α-methyl thio-Δ⁴-3-ketone III, and BMD hydrolysis with formic acid[13] furnished the desired 2α-methylthiocortisone (IV).

(1) 2-Alkylthio,[2-5] bis-alkylthio,[3] 7α-alkylthio,[5] and 21-alkylthio[6] steroid hormone derivatives have been reported.

(2) J. M. Krämer, K. Brückner, K. Irmscher, and K. Buck, *Chem. Ber.*, **96**, 2803 (1963).

(3) R. L. Clarke, *J. Org. Chem.*, **28**, 2026 (1963).

(4) (a) A. S. Hoffman, H. M. Kissman, and M. J. Weiss, *J. Med. Pharm. Chem.*, **5**, 962 (1962); (b) K. Takeda, T. Komeno, N. Tokutake, and Y. Kanemasu, *Chem. Pharm. Bull.* (Tokyo), **12**, 905 (1964).

(5) R. E. Schaub and M. J. Weiss, *J. Org. Chem.*, **26**, 3915 (1961).

(6) R. E. Schaub and M. J. Weiss, *ibid.*, **26**, 1223 (1961).

(7) The successful condensation of 2-hydroxymethylene-androstan-3-one with trimethylene dithiolene-*p*-thiosulfonate has been reported [J. L. Beton, T. G. Halsall, E. R. H. Jones, and P. C. Phillips, *J. Chem. Soc.*, 753 (1957); see also R. L. Autrey and P. W. Scullard, *J. Am. Chem. Soc.*, **87**, 3284 (1965)]. For a discussion of the reaction of sulfenyl halides with various nucleophiles see E. E. Reid, "Organic Chemistry of Bivalent Sulfur," Vol. I, Chemical Publishing Co., Inc., New York, N. Y., 1958, p 273 ff.

(8) J. F. Pilcher, G. R. Allen, Jr., and M. J. Weiss, *J. Med. Chem.*, **10**, 106 (1967).

(9) (a) H. Murozik, P. Buchschacher, J. Hannah, and J. H. Fried, *ibid.*, **7**, 584 (1964); (b) G. R. Allen, Jr., G. O. Morton, H. M. Kissman, and M. J. Weiss, to be published.

(10) See G. R. Allen, Jr., and M. J. Weiss, *J. Org. Chem.*, **27**, 4081 (1962), and references cited therein.

(11) The lithium salt gave similar results (see Experimental Section).

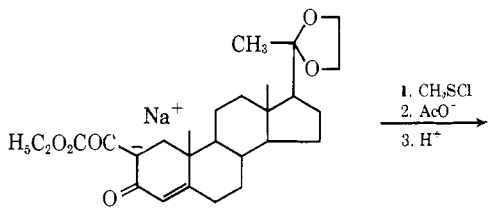
(12) C. E. Bublitz, L. I. Feldman, H. M. Kissman, and M. J. Weiss, *J. Org. Chem.*, **27**, 2122 (1962).

(13) R. E. Beyler, R. M. Moriarty, F. Hoffman, and L. H. Sarett, *J. Am. Chem. Soc.*, **80**, 1517 (1958).

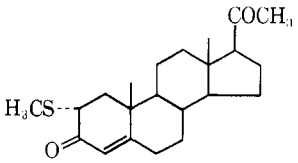
Although this compound caused an increase in sodium and urine excretion,[14] it was only less than one-half as active as hydrocortisone according to liver glycogen and thymolytic assays.[15] Inasmuch as 2α-methyl derivatives in the 11-carbonyl series show almost no corticoid activity in instances when the corresponding 11β-hydroxy derivatives are highly active,[16] it remained of interest to prepare the 2α-methylthio derivative VI of hydrocortisone. This compound was obtained from the cortisone BMD derivative III by 3-ketalization (to V), sodium borohydride reduction of the 11-carbonyl function, and acid-catalyzed deblocking of the side chain and the 3-carbonyl group. The α configuration is assigned to the 2-methylthio group in III–VI, as well as in VIII (below), on the supposition that the acetate-induced dealkoxalylation procedure, and also with respect to IV, VI, and VIII, the acid-catalyzed side chain deblocking procedure, will lead to the formation of the more stable equatorial derivatives.

2α-Methylthiohydrocortisone was inactive following single subcutaneous injections of 900 μg of compound/rat in an antiphlogistic and thymus involution assay.[17]

By a similar sequence 2α-methylthioprogesterone (VIII) was prepared from the sodium salt (VII) of 2-ethoxalylprogesterone 20-ketal.[18] In the subcutaneous Clauberg assay[19] for progestational activity, VIII was inactive at a total dose of 1 mg.



VII



VIII

Experimental Section

Melting points were taken on a Kofler micro hot stage and are corrected. Ultraviolet spectra were determined in methanol on a Cary recording spectrophotometer and infrared spectra (KBr disks) on a Perkin-Elmer spectrophotometer (Model 21). An ethanolic solution of $FeCl_3$ was used for the enol test. Solutions were dried ($MgSO_4$) and the solvents evaporated under reduced pressure. Optical rotations were determined at 25° in $CHCl_3$ at concentrations, unless otherwise noted, of 0.74–1.2%.

Methanesulfenyl chloride was prepared by the chlorination of methyl disulfide with SO_2Cl_2 according to Brintzinger and co-workers,[20] or more conveniently with chlorine according to Douglass.[21] When prepared by the latter method, the sulfenyl halide was distilled at 80–100 mm into a −10° trap. The orange liquid was weighed out as quickly as possible to avoid reaction with atmospheric moisture.

17α,20;20,21-Bismethylenedioxy-2-methoxalyl-2-methylthiopregn-4-ene-3,11-dione (II). A.—To a suspension of 976 mg (2 mmoles) of 17α,20;20,21-bismethylenedioxy-2-methoxalylpregn-4-ene-3,11-dione (see I)[12] in 10 ml of methanol was added 2 ml of a 1 *N* methanolic CH_3ONa solution. The resulting dark solution was concentrated to dryness and reconcentrated after addition of a small amount of dry, peroxide-free dioxane. The precipitate was suspended as well as possible in 20 ml of dioxane, and 230 mg of freshly prepared methanesulfenyl chloride in 3 ml of dioxane was added. The mixture was mixed thoroughly with a glass rod for a few minutes, by which time most of the solid had dissolved and the color had changed to light yellow. The filtered solution was concentrated to dryness and the crystalline residue was washed with water, dissolved (CH_2Cl_2), washed with more water, and dried, and the solvent was evaporated. The product was washed with ether to afford 797 mg of solid II, mp 190–203° (75% crude yield, negative enol test). A sample was recrystallized from methanol–CH_2Cl_2 and then from ether–CH_2Cl_2 to afford material with mp 223–225°; $[\alpha]D$ 0°; λ_{max} 244 mμ (ε 12,020); λ 5.75 (s), 5.86 (s), 5.92 μ (s).

Anal. Calcd for $C_{27}H_{34}O_9S$: C, 60.65; H, 6.42; S, 5.99. Found: C, 60.51; H, 6.47; S, 6.20.

B.—To a solution of 976 mg (2 mmoles) of the methoxalyl derivative (see I) in 65 ml of dry benzene was added, under N_2 through a syringe, 0.57 ml of a solution of butyllithium in heptane (approximately 3.4 *N*), and then also through a syringe 0.5 ml (a large excess) of freshly prepared methanesulfenyl chloride. After 20 min the mixture was washed several times with water, aqueous $NaHCO_3$, and water, and dried, and the solvent was evaporated. The residue was triturated with ether, collected by filtration, and washed with ether until free from color to afford 650 mg of II, mp 190–210° (62%, negative enol test) and with an infrared spectrum identical with that obtained from material prepared by procedure A.

17α,20;20,21-Bismethylenedioxy-2α-methylthiopregn-4-ene-3,11-dione (III).—A stirred suspension of 650 mg (1.2 mmoles) of II in 20 ml of methanol was refluxed under N_2 with 1 g of potassium acetate for 1 hr. The mixture was concentrated to dryness, and the residue was distributed in water–$CHCl_3$. The organic phase was washed with a little water, 1% aqueous KOH, and water, and then dried, and the solvent was evaporated. The residue was crystallized from methanol with activated charcoal to afford 310 mg (57%) of III, mp 196–200°. The analytical sample was prepared from material obtained in a similar experiment and was recrystallized several times from CH_2Cl_2–ether; mp 197–199°; $[\alpha]D$ +53.5°; λ_{max} 238 mμ (ε 13,660); λ 5.87 (s), 5.97 μ (s).

Anal. Calcd for $C_{24}H_{32}O_6S$: C, 64.27; H, 7.19; S, 7.15. Found: C, 64.31; H, 7.58; S, 7.07.

2α-Methylthiocortisone (17α,21-Dihydroxy-2α-methylthiopregn-4-ene-3,11,20-trione, IV).—A mixture of 448 mg (1 mmole) of the blocked compound III and 20 ml of 60% aqueous formic acid[13] was heated on the steam bath for 40 min and concentrated to dryness at 50°. The residue was dissolved in 30 ml of $CHCl_3$ and the solution was washed with water, $NaHCO_3$, and water, and dried, and the solvent was evaporated. An attempt to purify the residue (470 mg) on a silica gel column was unsuccessful, and the eluted material (390 mg) was columned on Celite (diatomaceous silica) from the system heptane–ethyl acetate–methanol–water (2:3:3:2).[22] The material was dissolved in 3 ml each of the lower and upper phase of this system and 6 g of Celite was added. The mixture was packed on top of a column which had been prepared from 100 g of Celite and 50 ml of the lower phase. The column [78 × 2 cm, holdback volume (HBV) 80 ml] was eluted with the upper phase and the effluent stream was monitored at 240 mμ. Two, partially overlapping, peaks were eluted in the second to fourth holdback volumes, and each peak was recolumned from the above system on separate columns. From the peak containing the less polar material there was

(14) This assay is based on the response of adrenalectomized male rats to a single, subcutaneous, 16-μg dose, as measured on a 5-hr urine collection.

(15) These subcutaneous assays were carried out by the procedure described by S. Bernstein, R. Littell, J. J. Brown, and I. Ringler, *J. Am. Chem. Soc.*, **81**, 4573 (1959).

(16) W. E. Dulin, B. J. Bowman, and R. O. Stafford, *Proc. Soc. Exptl. Biol. Med.*, **94**, 303 (1957).

(17) G. Tonelli, L. Thibault, and I. Ringler, *Endocrinology*, **79**, 463 (1966).

(18) H. M. Kissman, A. M. Small, and M. J. Weiss, *J. Am. Chem. Soc.*, **82**, 2312 (1960).

(19) This assay was carried out under the supervision of Dr. E. Shipley of the Endocrine Laboratories, Madison, Wis., according to the McPhail modification [M. K. McPhail, *J. Physiol.* (London), **83**, 145 (1934)].

(20) H. Brintzinger, K. Pfannstiel, H. Koddebusch, and K. E. Kling, *Chem. Ber.*, **83**, 87 (1950).

(21) I. B. Douglass, *J. Org. Chem.*, **24**, 2004 (1959).

(22) For a more detailed description of this technique, developed by C. Pidacks, see M. J. Weiss, R. E. Schaub, G. R. Allen, Jr., J. F. Poletto, C. Pidacks, R. B. Conrow, and C. J. Coscia, *Tetrahedron*, **20**, 357 (1964).

obtained by evaporation and crystallization from CH_2Cl_2–ether 75 mg (19%) of IV, mp 160–166°. The analytical sample (positive blue tetrazoleum α-ketol test) had mp 165–166°; $[\alpha]D$ +181°; λ_{max} 239 mμ (ε 15,420); λ 5.87 (s), 6.00 μ (s).

Anal. Calcd for $C_{22}H_{30}O_5S$: C, 65.00; H, 7.44; S, 7.89. Found: C, 64.81; H, 7.80; S, 7.76.

17α,20;20,21-Bismethylenedioxy-3-ethylenedioxy-2α-methylthio-11β-hydroxypregn-5-ene.—A mixture of 762 mg of III, 1.7 ml of ethylene glycol, and 35 mg of *p*-toluenesulfonic acid in 35 ml of benzene was stirred vigorously at reflux for 5 hr. The water formed was removed by means of a Dean–Stark tube. The cooled solution was diluted with benzene and was washed with aqueous $NaHCO_3$, saline, and water, dried, and concentrated to dryness. The resulting solid was triturated with ether and collected to furnish 671 mg (80%) of V, mp 232–240°, which was submitted directly to $NaBH_4$ reduction as follows.

A mixture of 790 mg of V, 18 ml of 2.5% NaOH solution, and 2.56 g of $NaBH_4$ in 65 ml of methanol was heated at reflux for 70 hr. The cooled solution was diluted with 200 ml of CH_2Cl_2 and washed with water, saline solution, and finally with water until neutral, and dried, and the solvent was evaporated. The residue was crystallized with ether–methanol and collected to furnish 586 mg (74%) of mp 200–204°. Recrystallization from methanol containing a drop of pyridine afforded the analytical sample, mp 202–204°, $[\alpha]D$ −59° (0.5%); the material had no significant absorption in the ultraviolet: λ_{max} 2.86, 9.10, 9.25, 10.58 μ.

Anal. Calcd for $C_{26}H_{38}O_5S$: C, 63.13; H, 7.75; S, 6.49. Found: C, 63.17; H, 8.02; S, 6.37.

11β,17α,21-Trihydroxy-2α-methylthiopregn-4-ene-3,20-dione (VI).—A solution of 292 mg of 17α,20;20,21-bismethylenedioxy-3-ethylenedioxy-2α-methylthio-11β-hydroxypregn-5-ene in 20 ml of 50% aqueous acetic acid was heated on the steam bath for 3.5 hr, and then evaporated to dryness. The crude product was partition chromatographed[22] on Celite diatomaceous earth using the heptane–ethyl acetate–methanol–water (60:40:17:4) partition system. The stationary phase was thoroughly mixed (0.5 ml/g) with Celite (200 g) and the mix was packed into a glass column. The reaction product was dissolved in 5 ml of the lower phase, mixed with 10 g of Celite, and packed on top of the column. The HBV was 330 ml and the V_m/V_s ratio was 3.14. The product VI was isolated from the fraction with a peak at 3.5 HBV, and recrystallized from ether to furnish 65 mg (27%); mp 122–124° (gas); $[\alpha]^{25}D$ +125°; λ_{max} 241 mμ (ε 15,000); λ_{max} 2.90, 5.83, 6.02, 6.16 μ.

Anal. Calcd for $C_{22}H_{32}O_5S$: C, 64.68; H, 7.90; S, 7.85. Found: C, 63.97; H, 8.15; S, 7.61.

2α-Methylthioprogesterone (2α-Methylthiopregn-4-ene-3,20-dione, VIII).—To a solution of 2.24 g (5 mmoles if pure) of the ethoxalyl derivative (see VII)[18] of 20-ethylenedioxypregn-4-en-3-one in 10 ml of methanol was added 5 ml of 1 *N* methanolic $NaOCH_3$. The dark red solution was concentrated to dryness at 40° and the residue was reevaporated with 20 ml of dry dioxane. This residue was mixed with 30 ml of dioxane (suspension) and 470 mg of methanesulfenyl chloride. The mixture was allowed to stand at room temperature 30 min. Most of the gel dissolved by the end of this period, but the mixture still gave a strong enol test. It was evaporated and the residue was redissolved in 100 ml of benzene. The solution was washed with water and then portionwise with 1% aqueous KOH until the yellow color was no longer extracted. The combined aqueous washings were acidified with dilute H_2SO_4, and the mixture was extracted ($CHCl_3$). The chloroform extracts were washed with water, dried, and concentrated to dryness to afford 876 mg of a yellow glass which gave a strong enol test. This material was not further investigated. The original benzene solution was washed with water and dried, and the solvent was evaporated. The residue was dissolved in 30 ml of methanol containing 2 g of potassium acetate, and the solution was allowed to reflux for 1 hr and was then concentrated to dryness. The residue was partitioned between water and benzene, and the organic phase was washed several times with KOH and then with water. The dried solution was concentrated to dryness and the partially crystalline residue (1 g) was redissolved in 25 ml of methanol containing 1 ml of 8% H_2SO_4. The solution was heated at reflux for 45 min, and was then cooled and neutralized with Duolite A-4 anion-exchange resin (OH form). The resin was removed by filtration and was washed thoroughly with methanol. The filtrate was evaporated of solvent and the residue was dried by evaporation with $CHCl_3$ to give an orange gum (0.9 g) which was dissolved in ether, treated with decolorizing charcoal, and evaporated.

Crystallization and recrystallization from ether afforded 162 mg (9%) of solid (VIII), mp 176–180°. The analytical sample, obtained in a similar experiment and recrystallized from ethyl acetate, had mp 180–183°; $[\alpha]D$ +202° (0.41%); λ_{max} 240 mμ (ε 12,060); λ 5.87 (s), 5.99 (s), 6.13 μ (m).

Anal. Calcd for $C_{22}H_{32}O_2S$: C, 73.29; H, 8.95; S, 8.45. Found: C, 73.22; H, 9.14; S, 8.76.

Acknowledgment.—We wish to thank Mr. W. Fulmor and staff for the spectrometric and polarimetric data, Mr. L. Brancone and staff for the microanalytical data, Mr. C. Pidacks and staff for partition chromatographic work, and Dr. G. Tonelli and staff for the biological assays.

Steroidal γ-Lactones. The Claisen Condensation of 17α-Acetate with 17β-Acetyl

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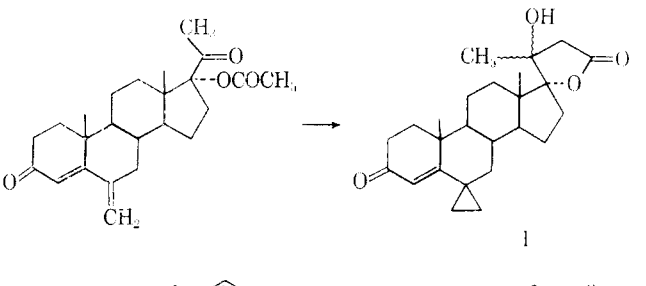
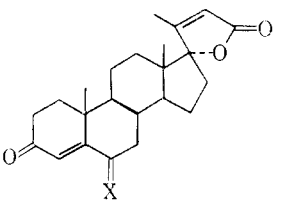
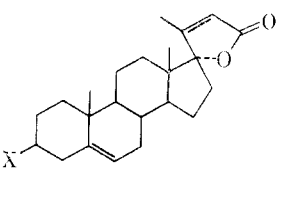
In attempting to prepare a 6-spirocyclopropyl steroid by the reaction of dimethylsulfoxonium methylide in the presence of excess sodium hydride with 6-methylene-17α-acetoxypregn-4-ene-3,20-dione,[1] the product was shown by analysis and determination of spectra to be 17α,20-dihydroxy-3-oxo-6-spirocyclopropyl-23-norchol-4-enic acid γ-lactone (I).



I



II, X = H, H
V, X =



III, X = HO
IV, X = CH_3COO

It appears that two reactions occurred, the 6-spiro cyclopropane formation by addition of dimethylsulfoxonium methylide to the conjugated unsaturation and the base-catalyzed condensation of the α position of the ester with the ketone of C-20.

Further examples, which limited the reaction only to the lactone formation, yielded the $\Delta^{20(22)}$-lactones (see Tables I and II). Thus the 17α-acetoxypregnene-20-

(1) D. Burn, G. Cooley, M. T. Davies, J. W. Ducker, B. Ellis, P. Feather, A. K. Hiscock, D. N. Kirk, A. P. Leftwick, V. Petrow, and D. M. Williamson, *Tetrahedron*, **20**, 597 (1964).