The reaction mixture was diluted with 75 ml. of chloroform; the solid was removed by filtration and rinsed with 25 ml. of chloroform. The solution was washed with four 25-ml. portions of water, dried over anhydrous sodium sulfate at 0°, and concentrated to dryness at reduced pressures. The residue was dried over sulfuric acid in a vacuum desiccator; yield 0.93 g., m.p. 194–195° (dec.), $\lambda_{max}^{ethanol}$ 238 mμ, log ε 3.12 (7.8% Δ⁴-3-ketone).

Anal. Calcd. for $C_{23}H_{31}BrO_6$: Br, 16.53. Calcd. for $C_{23}H_{31}ClO_6$: Cl, 8.08. Found: Br, 10.09, 10.11; Cl, 1.48, 1.53.

Reaction of 4-Bromo-17α-hydroxy-21-acetoxypregnane-3,11,20-trione with Sodium Iodide in Dimethylformamide.—A solution of 1.21 g. (2.5 millimoles) of the 4-bromo compound (Br, 15.61, 15.72) and 1.12 g. (7.5 millimoles) of anhydrous sodium iodide in 10 ml. of dimethylformamide was heated for 2 hours at 100° under nitrogen. Dilution of the deep-red reaction solution with 10 ml. of 5% sodium bisulfite solution precipitated a white solid; yield 0.64 g., m.p. 198–199° (dec.), $\lambda_{max}^{ethanol}$ 238 mμ, log ε 3.37 (14.4% Δ⁴-3-ketone).

Anal. Calcd. for $C_{23}H_{31}BrO_6$: Br, 16.53. Calcd. for $C_{23}H_{31}IO_6$: I, 23.93. Found: Br, 12.03; I, 0.91.

Dilution of the filtrate with 50 ml. of water precipitated more solid; yield 0.33 g., m.p. 205–206° (dec.), $\lambda_{max}^{ethanol}$ 238 mμ, log ε 3.09 (7.5% Δ⁴-3-ketone).

Anal. Calcd. for $C_{23}H_{31}BrO_6$: Br, 16.53. Calcd. for $C_{23}H_{31}IO_6$: I, 23.93. Found: Br, 13.63; I, 0.77.

Phase Distribution Study.[4]—The solubility of 4-bromo-17α-hydroxy-21-acetoxypregnane-3,11,20-trione [m.p. 195–196° (dec.), $[\alpha]_D$ +112 and 111°] was measured in diethyl ketone at 24° in the presence of varying quantities of solid phase. A plot of the ratios, mg. of solid/mg. of solvent *versus* mg. of solid/ml. of solution, resulted in a well-defined curve characteristic for a pure substance, the maximum solubility being 19.30 mg. per ml.

Acknowledgment.—The author expresses his appreciation for the coöperation of the Physics Department, particularly Dr. J. L. Johnson, W. A. Struck, H. Emerson and their assistants, and of all the members of our staff who have contributed materially to the subject matter of this report. The author thanks Doctors A. C. Ott and D. A. Shepherd for their continued interest, encouragement and guidance in connection with this work.

KALAMAZOO, MICHIGAN

[CONTRIBUTION FROM THE EASTERN REGIONAL RESEARCH LABORATORY][1]

Steroidal Sapogenins. VIII. Markogenin (22b-Spirostane-2ξ,3β-diol). A New Sapogenin Isolated from Yucca[2]

BY MONROE E. WALL, C. ROLAND EDDY, SAMUEL SEROTA AND ROBERT F. MININGER

RECEIVED MARCH 11, 1953

22b-Spirostane-2ξ,3β-diol, a new steroidal sapogenin, has been isolated from certain Yucca species. The structure of the compound has been determined from chemical and infrared data. The new sapogenin is distinctly different from the previously reported texogenin which is stated to have the same structure.

During a survey of sapogenaceous plants, a dihydroxy sapogenin, normal at carbon 22, was isolated from several *Yucca* species. Preliminary examination indicated that the compound was a new sapogenin. Chemical and infrared studies proved conclusively that the dihydroxy sapogenin had the structure assigned by Marker[3] to texogenin. However, as shown in Table I, a comparison of the melting points of the new sapogenin and its derivatives with those given by Marker, *et al.*, for texogenin, show obvious discrepancies. In particular the new sapogenin melts some 80° higher than "texogenin."

TABLE I

COMPARISON OF THE MELTING POINTS OF MARKOGENIN AND ITS DERIVATIVES WITH THOSE OF TEXOGENIN, AND SPECIFIC ROTATIONS OF THE NEW GENIN

	M.p., °C.	$[\alpha]^{25}D$ Chloroform
22b-Spirostane-2ξ,3β-diol	256–257	−70.3
Texogenin	172–175[3]	....
22b-Spirostane-2ξ,3β-diol 2,3-diacetate	185–186	−84.2
Texogenin acetate	172–173[3]	....
2,3-seco-22b-Spirostane-2//3-dioic acid	244–246	−46.8
Texogenic acid	268–269[3]	....

The method used by Marker, *et al.*,[3] for the isolation of texogenin was complex[4] in that it was necessary to separate the compound from six other sapogenins. In fact the entire history of texogenin is perplexing.[5]

In view of these facts we therefore wish to present the details of an unequivocal isolation and structure proof of the new steroidal sapogenin. In order to avoid confusion with texogenin, we have named the new sapogenin Markogenin.[6]

We have isolated Markogenin (22b-spirostane-2ξ,3β-diol) from the leaves of *Yucca faxoniana*, *Y. schidigera* and a yucca by-product leaf powder[7]

(1) One of the laboratories of the Bureau of Agricultural and Industrial Chemistry, Agricultural Research Administration, United States Department of Agriculture. This work was done as part of a coöperative arrangement between the Bur. of Plant Industry, Soils and Agricultural Engineering and Bur. of Agricultural and Industrial Chemistry of the U. S. Department of Agriculture and the Natl. Institute of Health, Department of Health, Education and Welfare. Article not copyrighted.

(2) Paper VII, M. E. Wall, *et al.*, *J. Am. Pharm. Assoc., Sci. Ed.*, in press.

(3) R. E. Marker, *et al.*, THIS JOURNAL, **69**, 2167 (1947).

(4) R. E. Marker, *et al.*, *ibid.*, **69**, 2226 (1947).

(5) Marker, *et al.*, *ibid.*, **69**, 2226 (1947), stated that texogenin was isolated by conversion to pseudotexogenin = pseudosamogenin (20(22)-furosten-2ξ,3β,26-triol), followed by acid isomerization back to texogenin. This was in contradiction to statements in another section of this paper, p. 2197, in which it was stated that pseudosamogenin reverts to samogenin. Marker, *et al.*, resolved this difficulty by assuming that some of the texogenin did not form the pseudo derivative and hence was recovered unchanged. Later, Marker and Lopez, *ibid.*, **69**, 2373 (1947), stated that acid isomerization of pseudosamogenin results in the formation of an 80-20 mixture of samogenin and texogenin, respectively. They concluded that *texogenin does not occur naturally* but is an artifact arising from samogenin. In a following paper, *ibid.*, **69**, 2383 (1947), Marker and Lopez treat texogenin = neosamogenin as a naturally occurring product. Consequently the status of this sapogenin is indeed dubious.

(6) In honor of Russell Marker.

(7) Anonymous, *Chem. Eng. News*, **30**, 2822 (1952).

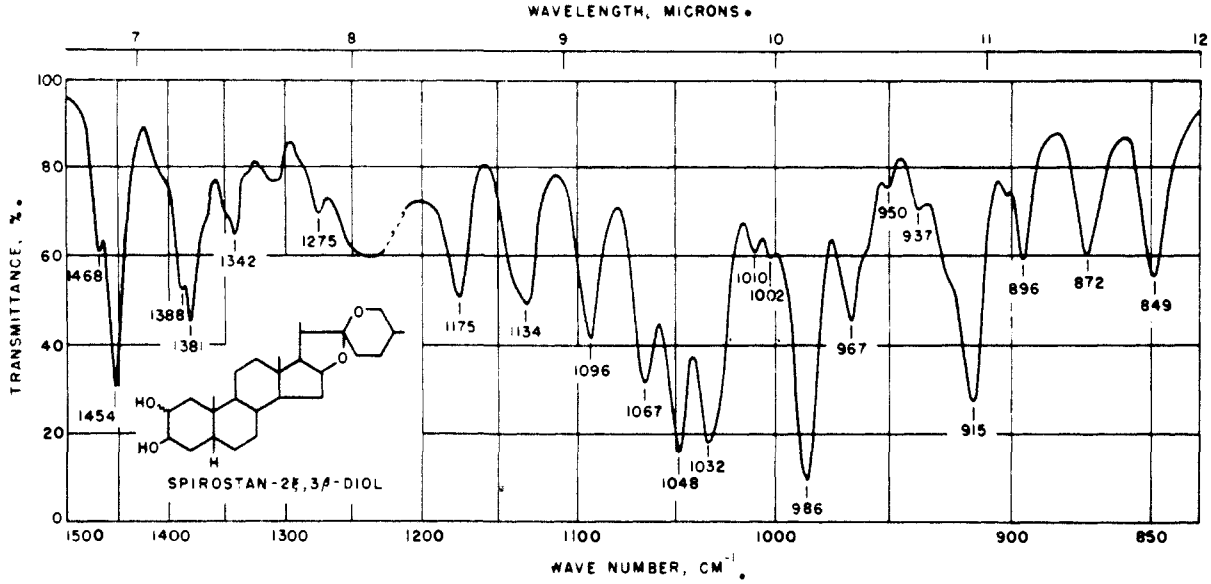


Fig. 1.—22b-Spirostane-2ξ,3β-diol, 20 g. per l. in chloroform, 0.50-mm. cell.

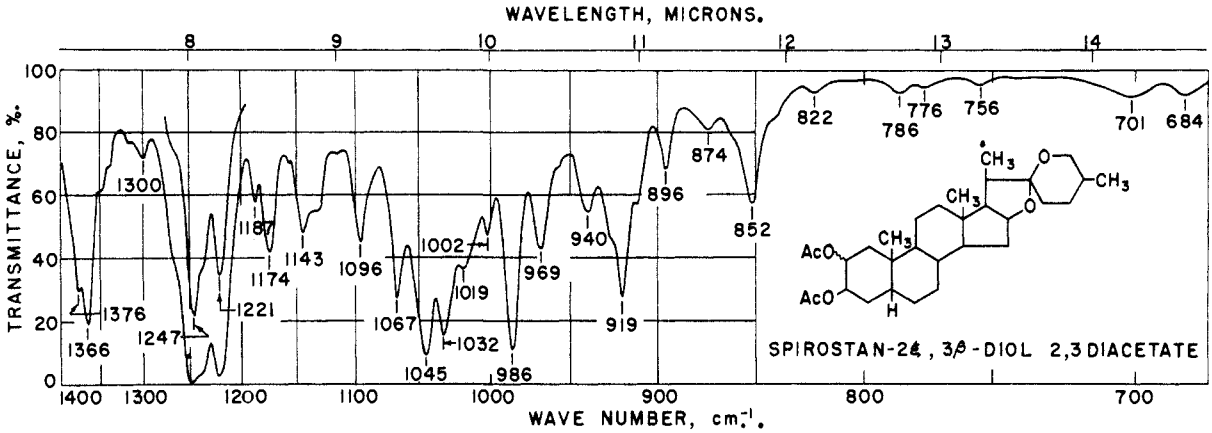


Fig. 2.—22b-Spirostane-2ξ,3β-diol 2,3-diacetate, 10 g. per l. in carbon disulfide, 1.1-mm. cell. The upper curve is at 3.0 g. per l. in the same cell.

believed to be derived from *Y. baccata*. We have also found it in the fruiting pods of *Y. schidigera*. In every case sarsasapogenin (22b-spirostan-3β-ol) was also present, but no other sapogenins were found. The biogenetic relationship is apparent, and similar findings have been reported by Marker, *et al.*,[3] for other sapogenins. Rather surprisingly in the case of texogenin, it was not associated with sarsasapogenin, although six other sapogenins were reported.[3]

Preliminary indications of the structure of markogenin were given by the following evidence: (1) The adsorption properties indicated that the compound contains two or more hydroxyl groups, since it is tenaciously adsorbed by alumina (and other adsorbents) and can be quantitatively separated in this manner from the accompanying 22b-spirostan-3β-ol (sarsasapogenin).[8] (2) Oxidation with cold chromic acid located the hydroxyls as probably at positions 2 and 3, since this reaction produces a 2//3-dioic acid,[9] a compound soluble in aqueous alkali which can be reprecipitated by acidification. (3) The infrared spectrum between 850 and 1000 cm.$^{-1}$ indicated that the compound has the normal (22b) configuration.[10,11] The sapogenin in chloroform has typical F-ring absorption bands at 849, 896, 915 and 986 cm.$^{-1}$ (Fig. 1), with the 915 band much stronger than the 896. The diacetate has a similar system of bands (Fig. 2). (4) The strength of the acetate absorption bands at 1221 and 1247 cm.$^{-1}$ indicated that the sapogenin contains two hydroxyl groups; and the 1221 cm.$^{-1}$ band, which is characteristic[10] of samogenin acetate (22a-spirostane-2ξ,3β-diol diacetate), suggests that the hydroxyls are at positions 2 and 3, with a *cis* fusion of the A and B rings. (5) The infrared spectrum indicates absence of unsaturation or carbonyl groups, since no absorption bands are found in the 800–850 cm.$^{-1}$ and 1600–1800 cm.$^{-1}$ regions.

This evidence indicated that markogenin has the structure 22b-spirostane-2ξ,3β-diol. Confirmation of this structure was obtained by isomerization of the F-ring, by prolonged refluxing of markogenin

(8) M. E. Wall, M. M. Krider, E. S. Rothman and C. R. Eddy, *J. Biol. Chem.*, **198**, 533 (1952).

(9) Reference 3, p. 2176.

(10) C. R. Eddy, M. E. Wall and M. K. Scott, *Anal. Chem.*, **25**, 266 (1953).

(11) M. E. Wall, C. R. Eddy, M. L. McClennan and M. E. Klumpp, *ibid.*, **24**, 1337 (1952).

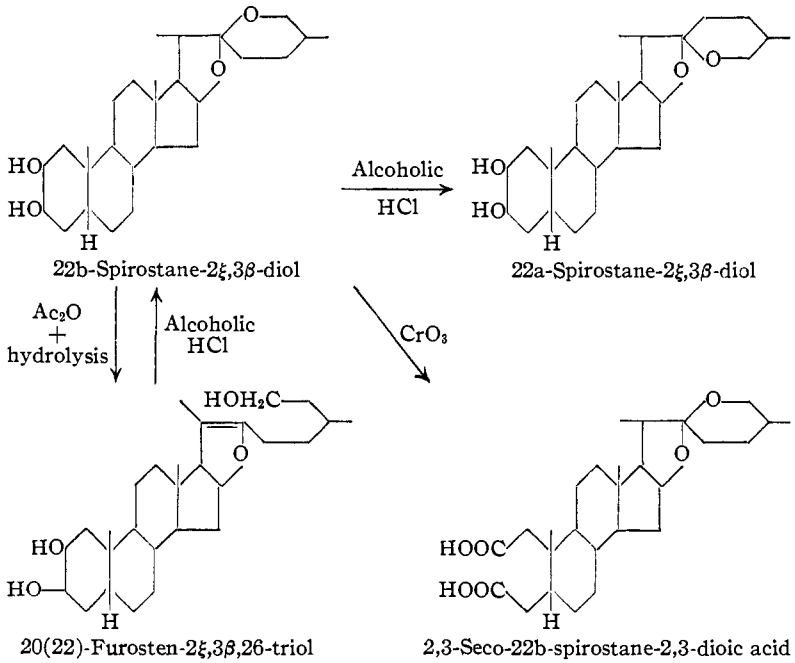
with alcoholic hydrochloric acid, to give a compound whose physical properties and infrared spectrum were essentially identical with those of the samogenin sample isolated by us.

Further confirmation of the close relationship of markogenin to sarsasapogenin was obtained from the acid isomerization of the pseudomarkogenin and pseudosamogenin. On short refluxing with alcoholic hydrochloric acid the former produced only markogenin and the latter samogenin. The reversion of pseudomarkogenin to markogenin on acid isomerization is quite analogous to previously recorded observations[12] which show that pseudosarsasapogenin forms sarsasapogenin when treated with acid. The behavior of pseudosamogenin which reverts to samogenin is not analogous to Marker's earlier work[12] but is more in accord with later findings of Marker and Lopez.[5]

The reaction sequences described above are shown in the following diagram and firmly establish the structure of markogenin as 22b-spirostane-2ξ,3β-diol.

The data suggest that pseudomarkogenin and pseudosamogenin cannot be the same since these compounds on acid isomerization form different sapogenins. This is in contrast with data reported by Marker and co-workers for pseudosarsasapogenin and pseudosmilagenin[13,14] and for pseudosamogenin and pseudotexogenin[5] indicating that each pair of pseudo compounds were identical. We are engaged in more definitive researches to prove this latter point.

The only uncertain feature in the structure of markogenin is the steric configuration of the hydroxyl group on carbon 2. By analogy the configuration is probably 2α,[14] although this is not certain.



Experimental

Isolation of Spirostan-2ξ,3β-diol.—Dry plant powder was extracted with boiling 80% ethanol; fresh material with boiling 95% ethanol. Preliminary purification, acid hydrolysis, and adsorption on activated alumina were carried out as previously described.[8] Only sarsasapogenin and the new steroid were found, and the separation of monohydroxy from the new dihydroxy sapogenin was clean cut, the former being eluted with 10% chloroform in benzene and the latter with 10–20% ethanol in benzene. Table II presents data on the plant species, total sapogenin content and percentages of the new sapogenin found.

TABLE II

22b-SPIROSTANE-2ξ,3β-DIOL CONTENT OF VARIOUS YUCCA SPECIES

Species	Plant part	Total sapogenin, % MFB	22b-Spirostane-2ξ,3β-diol, % total
Yucca baccata[a]	Leaf	1.0	20
Yucca faxoniana[b]	Leaf	0.5	60
Yucca schidigera[c]	Leaf	1.3	45
Yucca schidigera[d]	Fruiting pod	3.3	25.5

[a,c,d] Obtained by Dr. H. S. Gentry, Bur. of Plant Industry, Soil and Agricultural Engineering, ERRL numbers 0-1686B, S-2608 and S-2777, respectively. [b] ERRL 0-1678 obtained through the courtesy of Dr. Max Tishler, Merck and Company.

Melting points were made with a Kofler micro melting point apparatus. Optical rotations were conducted in chloroform. Samples were dried 48 hours at 100° *in vacuo* prior to analysis.

22b-Spirostane-2ξ,3β-diol was crystallized from methanol, m.p. 256–257°, $[\alpha]^{25}D$ −70.3°. The infrared spectrum is shown in Fig. 1. *Anal.* Calcd. for $C_{27}H_{44}O_4$: C, 74.95; H, 10.25. Found: C, 74.88; H, 10.48.

22b-Spirostane-2ξ,3β-diol 2,3-diacetate was crystallized from methanol, m.p. 185–186°, $[\alpha]^{25}D$ −84.2. The infrared spectrum is shown in Fig. 2. *Anal.* Calcd. for $C_{31}H_{48}O_6$: C, 72.07; H, 9.37. Found: C, 72.25; H, 9.47.

Markogenic Acid, 2,3-seco-22b-Spirostane-2,3-dioic Acid.—Markogenin (22b-spirostane-2ξ,3β-diol) 0.5 g. was dissolved in 35 ml. of glacial acetic acid. Chromic acid, 0.8 g., in 8 ml. of 80% acetic acid was added and the mixture allowed to stand at room temperature (26°) for 30 minutes. After dilution with water, the sample was extracted with ether and the ether washed with dilute sodium chloride solution followed by several water washes. The ethereal solution was then extracted three times with 5% sodium hydroxide. The alkaline solution was acidified and 0.35 g. of crude dioic acid obtained. After several recrystallizations from ether the compound had a m.p. 244–246°, $[\alpha]^{25}D$ −46.8°. The infrared spectrum in chloroform (from 800 to 1400 cm.$^{-1}$) showed many of the bands found in the original markogenin, including the usual four bands associated with normal configuration of the F-ring. Otherwise, the spectrum was essentially what would be expected from removal of alcoholic groups and addition of carboxyl groups.

Anal. Calcd. for $C_{27}H_{42}O_6$: C, 70.10; H, 9.15; neut. equiv., 231.3. Found: C, 69.91; H, 9.25; neut. equiv., 239.8.

Acid isomerization of markogenin to samogenin: Markogenin, 0.5 g., was dissolved in a solution of 85 ml. of absolute ethanol and 15 ml. of concentrated hydrochloric acid and the solution refluxed 48 hours. After dilution with water and extraction with ether followed by the usual work up, 0.3 g. of crude product was obtained. On crystallization from methanol, samogenin, m.p. 194–197°, was obtained. The infrared spectrum of this compound

(12) R. E. Marker, *et al.*, THIS JOURNAL, **62**, 896 (1940).
(13) R. E. Marker, *et al.*, *ibid.*, **61**, 3592 (1939); **62**, 518, 648 (1940).
(14) J. Pataki, G. Rosenkranz and C. Djerassi, *ibid.*, **73**, 5375 (1951).

was compared with that of a sample of samogenin isolated at this Laboratory. The spectra were essentially identical, the only significant difference being that the ratio of the absorbances of the 897 and 916 cm.$^{-1}$ bands was not so great in the isomerized product as in the authentic samogenin. This probably indicates that conversion was not quite complete and that a small amount of the original normal sapogenin remained unreacted.

Samogenin (22a-Spirostane-2ξ,3β-diol).—A sample of the inflorescent buds of *Yucca carnerosana* was extracted with ethanol as described under markogenin. After standard purification and acid hydrolysis,[8] the crude sapogenin was chromatographed on activated alumina. A minor fraction was eluted with benzene and was identified as smilagenin in our usual manner.[8,10] The major component, 75% of the total sapogenin content (which was 0.4% on a moisture-free basis) was eluted with 20% ethanol in benzene. After evaporating the solvent and several recrystallizations from methanol, samogenin, m.p. 203–205, $[\alpha]^{25}D$ −74°, diacetate m.p. 198°, $[\alpha]^{25}D$ −84° was isolated. The infrared spectra of our samogenin and that from a sample supplied by Marker were compared at Sloan-Kettering Institute by Dr. Thomas A. Gallagher and were found to be identical.

Effect of Acid Isomerization on Pseudomarkogenin and Pseudosamogenin.—Markogenin diacetate, 1.0 g., was heated in a sealed carius tube with 10 ml. of acetic anhydride for ten hours at 200°. After cooling, a small quantity of solids was removed by filtration. The pseudotriacetate was extracted from the acetic anhydride by repeated extraction with hexane. The hexane solution was washed with sodium bicarbonate solution and distilled water. After drying over anhydrous sodium sulfate, the solvent was evaporated. A yield of 0.92 g. of a viscous, pale yellow oil was obtained. Infrared examination of this product in carbon disulfide indicated that the sapogenin peaks associated with the spiroketal grouping in sapogenins were essentially absent.[11] The crude triacetate, (20-(22)-furosten-2ξ,3β,26-triol triacetate) was refluxed one hour with a solution of 5% potassium hydroxide in methanol. After dilution with water and ether extraction, 0.6 g. of crude pseudotriol was obtained. A solution consisting of 0.16 g. crude triol, 1 ml. of concentrated hydrochloric acid and 10 ml. of methanol was refluxed one hour. On dilution with water and ether extraction crude markogenin was obtained. The infrared spectrum of the crude product in carbon disulfide was essentially identical with that of an authentic sample of markogenin. The crude markogenin was crystallized once from ether and twice from methanol; m.p. 255–257°. There was no depression of m.p. when mixed with an authentic sample.

Samogenin diacetate, 0.38 g., was converted to the pseudogenin and hydrochloric acid isomerized as described above for markogenin. The infrared spectra of the crude product were essentially identical with those of an authentic sample of samogenin. The crude samogenin was crystallized once from ether and twice from methanol, m.p. 202–203°. There was no depression of m.p. when mixed with an authentic specimen.

Infrared spectra were obtained with a Beckman model IR-3 spectrophotometer, using sodium chloride prisms. Carbon disulfide (A.C.S. grade) was used as received. Chloroform (A.C.S. grade) was purified by filtration through activated alumina and distillation from lithium aluminum hydride.

Acknowledgment.—We gratefully acknowledge the assistance of Mary-Anne Morris, Mary Klumpp Scott and Howard W. Jones with the infrared work. We wish to express our appreciation to Dr. Thomas A. Gallagher, of Sloan-Kettering Institute, for comparing our sample of samogenin with a sample isolated by Marker. We thank Mrs. Ruth B. Kelley for the carbon and hydrogen analyses.

PHILADELPHIA, PA.

[JOINT CONTRIBUTION FROM THE RESEARCH LABORATORIES OF SYNTEX, S.A., AND THE INSTITUTO DE QUIMICA DE LA UNIVERSIDAD NACIONAL AUTONOMA DE MEXICO]

Steroids. XLVIII.[1] 19-Norprogesterone, a Potent Progestational Hormone

BY CARL DJERASSI,[2] L. MIRAMONTES AND G. ROSENKRANZ

RECEIVED APRIL 15, 1953

Reduction of 3-methoxy-17-acetyl-1,3,5-estratriene with lithium in liquid ammonia leads to $\Delta^{2,5(10)}$-19-nor-3-methoxy-20-hydroxypregnadiene, which upon acid hydrolysis and chromium trioxide oxidation affords 19-norprogesterone. This substance exhibits four to eight times the biological activity of progesterone in rabbits.

Progestational activity is extremely specific and is limited to the natural hormone, progesterone, and a few of its dehydro derivatives.[3] As a result, it appeared very surprising that a substance, believed to be 19-norprogesterone, exhibited progestational activity[4] equal to that of the parent hormone. This 19-norprogesterone was obtained[5] as a resin in 0.07% yield by a twelve-step degradation from strophanthidin, and was clearly a mixture of stereoisomers with the "abnormal" configuration at C-14 (β) and C-17 (α),[6,7] and of unknown configuration at C-10. Since 17-isoprogesterone[8] and 14-iso-17-isoprogesterone[6] show no progestational activity, there remains the intriguing alternative that removal of the angular methyl group confers high biological potency. That this does not necessarily follow, at least in the androgen series, is demonstrated by the fact that 19-nortestosterone,[9,10] which possesses the "natural" configuration at all asymmetric centers,[10,11] exhibits only *ca.* one-third the androgenic activity of testosterone.[10-12] Since the mechanism of androgenic and progestational activity is not necessarily comparable, it appeared of very considerable interest to synthesize 19-norprogesterone with the "natural"

(1) Paper XLVII, THIS JOURNAL, **75**, in press (1953).

(2) Department of Chemistry, Wayne University, Detroit 1, Michigan.

(3) *Cf.* M. Ehrenstein, *Chem. Revs.*, **42**, 457 (1948).

(4) W. M. Allen and M. Ehrenstein, *Science*, **100**, 251 (1944).

(5) M. Ehrenstein, *J. Org. Chem.*, **9**, 435 (1944).

(6) P. A. Plattner, H. Heusser and A. Segre, *Helv. Chim. Acta*, **31**, 249 (1948).

(7) M. Ehrenstein, G. W. Barber and M. W. Gordon, *J. Org. Chem.*, **16**, 355 (1951). Recently, Ehrenstein (*Chimia*, **6**, 287 (1952)), reported the isolation of a crystalline 19-nor-14-iso-17-isoprogesterone (with unknown configuration at C-10) from strophanthidin. Its biological activity has not yet been recorded, but, as was expected, the physical constants of this isomer are quite different from those of the presently described 19-norprogesterone (IIIc).

(8) A. Butenandt, J. Schmidt-Thomé and H. Paul, *Ber.*, **72**, 1112 (1939).

(9) A. J. Birch, *J. Chem. Soc.*, 367 (1950).

(10) A. L. Wilds and N. A. Nelson, THIS JOURNAL, in press.

(11) A. J. Birch and H. Smith, *J. Chem. Soc.*, 1882 (1951).

(12) A. J. Birch, *Ann. Repts. Prog. Chem.* (*Chem. Soc. London*), **47**, 210 (1950).