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

Steroidal Sapogenins. XIII.[1] $\Delta^{8(9)}$-22-Isospirosten-3α-ol

BY CARL DJERASSI, R. YASHIN AND G. ROSENKRANZ

Catalytic hydrogenation in a neutral or alkaline medium of Δ^4-22-isospirosten-3-one (I), $\Delta^{4,6}$-22-isospirostadien-3-one (IV) or $\Delta^{1,4,6}$-22-isospirostatrien-3-one (V) yielded predominantly the 5-*normal* isomer, 22-isospirostan-3-one (II), which afforded 22-isospirostan-3α-ol upon reduction with lithium aluminum hydride. A similar reaction sequence with $\Delta^{4,6,8(9)}$-22-isospirostatrien-3-one (VI) led by way of $\Delta^{8(9)}$-22-isospirosten-3-one (VII) to $\Delta^{8(9)}$-22-isospirosten-3α-ol (VIII), the first $\Delta^{8(9)}$-mono-unsaturated steroid with the *normal* configuration at C-5.

The synthesis of 11-oxygenated steroids from ring C unsubstituted starting materials has so far only been accomplished[2-4] by way of $\Delta^{7,9(11)}$-dien-3-ols. An alternate approach, which appears attractive, would be the study of reagents, known to attack the allylic position, upon $\Delta^{8(9)}$-unsaturated precursors. Such compounds are among the rarest and least studied steroids; aside from the naturally occurring yeast sterols, zymosterol, ascosterol and fecosterol, the only source for such substances is the catalytic hydrogenation[5] of $\Delta^{6,8(9)}$-cholestadien-3β-ol (isodehydrocholesterol) and $\Delta^{8(9),14}$-ergostadien-3β-ol (dehydroergostenol). No $\Delta^{8(9)}$-unsaturated representative is known in the androstane, pregnane, sapogenin or bile acid series. All of the above mentioned $\Delta^{8(9)}$-stenols possess the 3β-hydroxyallo configuration and the most accessible one, zymosterol ($\Delta^{8(9),24}$-cholestadien-3β-ol) (IX), has recently been examined[6] with negative results as a possible intermediate for the synthesis of 11-oxygenated steroids. In spite of this negative finding, it was considered desirable in this Laboratory to develop a synthetic route to $\Delta^{8(9)}$-unsaturated sapogenins with the *normal* configuration at C-5 for the following reasons: (a) No $\Delta^{8(9)}$-mono-unsaturated steroid with the 5β-configuration is known[6a]; (b) since 3α,9α-epoxides[7] are extremely useful in the synthesis of 11-oxygenated steroids and can be formed only from precursors possessing the 3α-hydroxy-5β-configuration, it is conceivable that $\Delta^{8(9)}$-unsaturated 3α-hydroxy-5β-steroids may behave quite differently than zymosterol (IX),[6] a $\Delta^{8(9)}$-3β-stenol of the *allo* series; (c) sapogenins can be degraded in two steps to pregnane derivatives and thus represent ideal starting materials for the hitherto unknown $\Delta^{8(9)}$-pregnenes. The present paper deals with the successful preparation of such a sapogenin, $\Delta^{8(9)}$-22-isospirosten-3α-ol (VIII).[8]

We have recently described the synthesis of certain 22-isospirostatrien-3-ones,[9] among them $\Delta^{4,6,8(9)}$-22-isospirostatrien-3-one (VI). Since Barton and Cox[5] have demonstrated that the 8,9-double bond is not hydrogenated in a neutral medium, the trienone VI appeared to be a suitable starting material for the preparation of the desired $\Delta^{8(9)}$-spirosten. Δ^4-22-Isospirosten-3-one (I) has been reported[10] to yield predominantly the 5-*normal* isomer 22-isospirostan-3-one (smilagenone) (II) upon hydrogenation with palladium–barium sulfate catalyst in ether solution. The same result was now obtained using palladized charcoal an ethanol, with or without added potassium hydroxide.[11] The presence of double bonds in the Δ^1- or Δ^6-positions did not change the steric course of the hydrogenation, since both $\Delta^{4,6}$-22-isospirostadien-3-one (IV)[12] and $\Delta^{1,4,6}$-22-isospirostatrien-3-one (V)[9] afforded 22-isospirostan-3-one (II) in essentially the same yield. As was to be anticipated by analogy to the results in the coprostan-3-one and cholestan-3-one series,[13] lithium aluminum hydride reduction of 22-isospirostan-3-one (II) led almost exclusively to 22-isospirostan-3α-ol (epismilagenin) (IIIa), which gave no precipitate with digitonin. In contrast, the Meerwein–Ponndorff reduction[10] yields as the major product the 3β-epimer (smilagenin).

Based on the above results, it appeared quite likely that hydrogenation of $\Delta^{4,6,8(9)}$-22-isospirostatrien-3-one (VI) with palladized charcoal in ethanol solution would lead to the desired $\Delta^{8(9)}$-22-isospirosten-3-one (VII), which in fact proved to be the case; a slightly higher yield was realized in the presence of potassium hydroxide. In concordance with that structure, lithium aluminum hydride reduction afforded $\Delta^{8(9)}$-22-isospirosten-3α-ol (VIIIa) as was to be expected[13] from a 3-ketosteroid with the normal configuration at C-5. The alcohol VIIIa gave no precipitate with digitonin, exhibited a yellow color with tetranitromethane and reacted rapidly with osmium tetroxide in ether solution. This latter behavior is typical[5] of $\Delta^{8(9)}$-unsaturated steroids and serves to distinguish them from the corresponding $\Delta^{7(8)}$ and $\Delta^{8(14)}$-isomers. Further work with $\Delta^{8(9)}$-spirostens is in progress, particularly that dealing with reactions of the 8,9-double bond and the degradation of the side chain.

(1) Paper XII, J. Pataki, G. Rosenkranz and C. Djerassi, THIS JOURNAL, **73**, 5375 (1951).

(2) E. M. Chamberlain, W. V. Ruyle, A. E. Erickson, J. M. Chemerda, L. M. Aliminosa, R. L. Erickson, G. E. Sita and M. Tishler, *ibid.*, **73**, 2396 (1951).

(3) L. F. Fieser, J. E. Herz and W. Huang, *ibid.*, **73**, 2397 (1951).

(4) G. Stork, J. Romo, G. Rosenkranz and C. Djerassi, *ibid.*, **73**, 3546 (1951); C. Djerassi, O. Mancera, G. Stork and G. Rosenkranz, *ibid.*, **73**, 4496 (1951).

(5) D. H. R. Barton and J. D. Cox, *J. Chem. Soc.*, 214 (1949).

(6) W. J. Adams, V. Petrow and R. Royer, *ibid.*, 678 (1951).

(6a) A. Windaus and G. Zühlsdorff, *Ann.*, **536**, 204 (1938), may have prepared $\Delta^{8(9)}$-coprosten-3β-ol, but the evidence is equivocal.

(7) E. C. Kendall and co-workers, *J. Biol. Chem.*, **162**, 571 (1946); *ibid.*, **164**, 569 (1946).

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

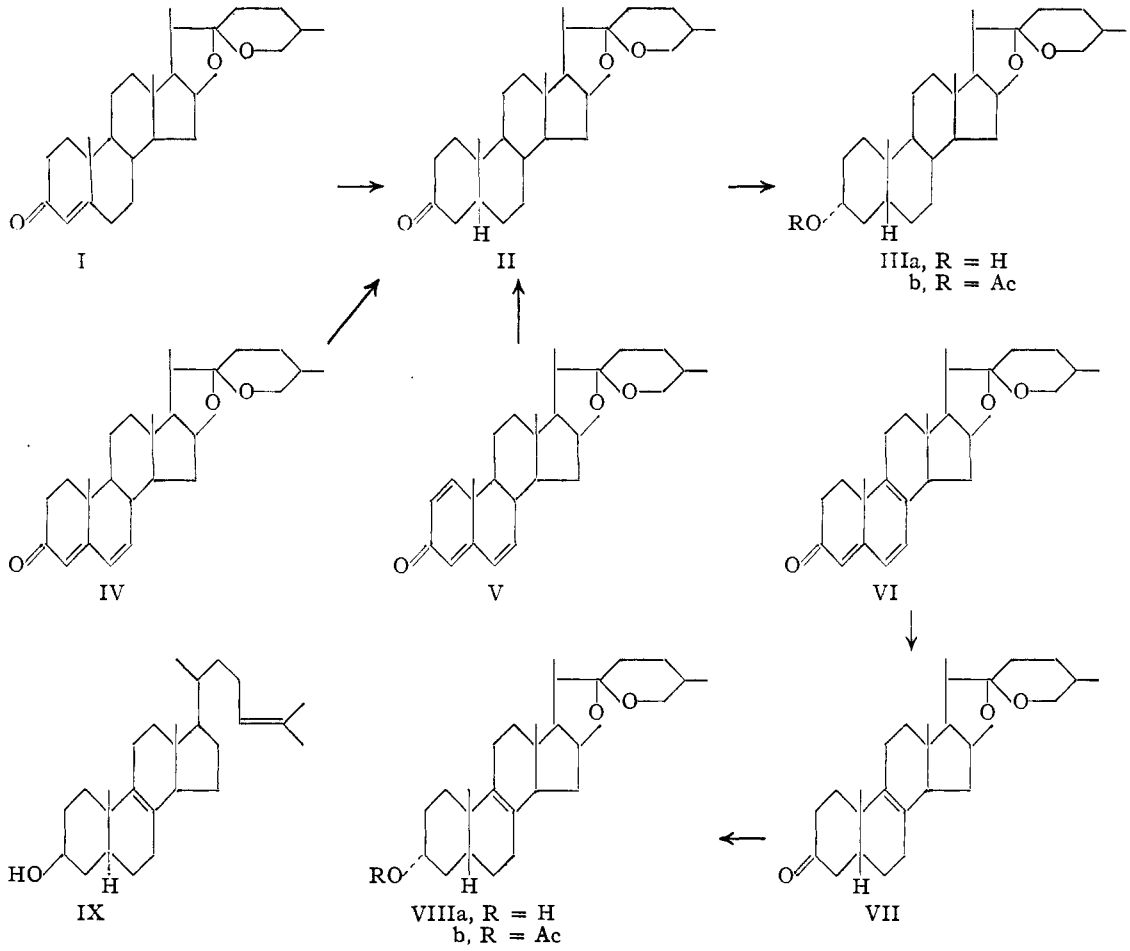
(9) R. Yashin, G. Rosenkranz and C. Djerassi, THIS JOURNAL, **73**, 4564 (1951).

(10) R. E. Marker, T. Tsukamoto and D. L. Turner, *ibid.*, **62**, 2525 (1940).

(11) At times, alkali increases the proportion of *cis*-isomer (*cf.* A. L. Wilds, J. A. Johnson and R. E. Sutton, *ibid.*, **72**, 5524 (1950), for leading references). P. L. Julian, "Recent Advances in Hormone Research," Vol. VI, Academic Press, New York, 1951, has studied in detail the effect of alkali upon the hydrogenation of Δ^4-3-ketosteroids.

(12) R. E. Marker and D. L. Turner, *ibid.*, **63**, 771 (1941); J. Romo, H. J. Ringold, G. Rosenkranz and C. Djerassi, *J. Org. Chem.*, **16**, Dec. (1951).

(13) C. W. Shoppee and G. H. R. Summers, *J. Chem. Soc.*, 687 (1950).



Experimental[14]

22-Isospirostan-3-one (II) (a) From Δ^4-22-Isospirosten-3-one (I).—A solution of 10.0 g. of Δ^4-22-isospirosten-3-one (I)[10] in 450 cc. of ethanol (distilled over Raney nickel catalyst) was shaken at room temperature and atmospheric pressure (580 mm.) in an atmosphere of hydrogen with 0.8 g. of prereduced 10% palladized charcoal catalyst (American Platinum Works, Newark, N.J.) for 35 minutes, at which time the hydrogen up-take, corresponding to one mole, had ceased. After pouring into water, collecting the precipitate and recrystallizing from chloroform–methanol there was obtained 7.2 g. of 22-isospirostan-3-one with m.p. 179–183°. The yield was not changed when the hydrogenation was carried out in the presence of 3.0 g. of potassium hydroxide. The analytical sample exhibited m.p. 186–188°,[15] $[\alpha]^{20}D$ −52°, ultraviolet absorption maximum at 284 mμ (log ϵ 1.7), infrared carbonyl band at 1718 cm.$^{-1}$, typical of saturated 3-ketosteroids.[16]

Anal. Calcd. for $C_{27}H_{42}O_3$: C, 78.21; H, 10.21. Found: C, 78.25; H, 10.27.

(14) All melting points are uncorrected. Rotations were determined in chloroform, ultraviolet absorption spectra in 95% ethanol and infrared spectra (Perkin-Elmer model 12C spectrometer with sodium chloride prism) in carbon disulfide solution. We are grateful to Srta. Paquita Revaque and staff for these measurements and to Srta. Amparo Barba of our microanalytical department for the analyses.

(15) The literature value (G. A. R. Kon, H. R. Soper and A. M. Woolman, *J. Chem. Soc.*, 1201 (1939)) is m.p. 187–189°. The isomeric 22-isoallospirostan-3-one is reported (ref. 10) to possess m.p. 202–205°, no rotation given. In our hands, 22-isoallospirostan-3-one (tigogenone), prepared by chromium trioxide oxidation of 22-isoallospirostan-3β-ol (tigogenin) isolated from *Agave parviflora*, showed m.p. 201–203°, $(\alpha)^{20}D$ −55°. The infrared spectra of the two isomers differed very considerably and thus were employed for purposes of identification.

(16) R. N. Jones, P. Humphries and K. Dobriner, THIS JOURNAL, **72**, 956 (1950).

(b) From $\Delta^{4,6}$-22-Isospirostadien-3-one (IV).[12]—The hydrogenation of the dienone IV was carried out as above, but adding 3.0 g. of potassium hydroxide prior to hydrogenating. 22-Isospirostan-3-one (II)[17] was isolated in 88% yield, while only 50–60% was obtained in the absence of potassium hydroxide.

(c) From $\Delta^{1,4,6}$-22-Isospirostatrien-3-one (V).[9]—The hydrogenation of the trienone V required 45 to 60 minutes for the consumption of three moles of hydrogen. With alkali, the yield of 22-isospirostan-3-one (II) was 65%, while without potassium hydroxide, it was lowered to 50%.

22-Isospirostan-3α-ol (IIIa).—A solution of 7.2 g. of 22-isospirostan-3-one (II) in 850 cc. of absolute ether was added slowly to a mixture of 7 g. of lithium aluminum hydride in 250 cc. of ether. After refluxing for one-half hour, the excess reagent was decomposed with acetone, the ether solution was washed well with dilute acid and evaporated; yield 7.0 g., m.p. 199–204°, $[\alpha]^{20}D$ −48°. Several recrystallizations from methanol–chloroform afforded 5.26 g. of the pure alcohol IIIa with m.p. 218–220°, $[\alpha]^{20}D$ −42°, which gave no precipitate with an alcoholic solution of digitonin. The 3β-epimer (smilagenin) is reported[18] to possess the following constants: m.p. 183–184°, $[\alpha]^{25}D$ −69°.

Anal. Calcd. for $C_{27}H_{44}O_3$: C, 77.83; H, 10.65. Found: C, 77.89; H, 10.76.

The acetate IIIb after recrystallization from methanol–ethyl acetate exhibited m.p. 162–164°, $[\alpha]^{20}D$ −33°.

Anal. Calcd. for $C_{29}H_{46}O_4$: C, 75.94; H, 10.11. Found: C, 76.28; H, 10.37.

$\Delta^{8(9)}$-22-Isospirosten-3-one (VII).—The hydrogenation of 1.0 g. of $\Delta^{4,6,8(9)}$-22-isospirostatrien-3-one (VI)[9] in 50 cc. of ethanol with 0.1 g. of 10% palladized charcoal and 0.3 g.

(17) D. H. R. Barton, J. D. Cox and N. J. Holness, *J. Chem. Soc.*, 1771 (1949), isolated Δ^{22}-coproergosten-3-one in the neutral hydrogenation of $\Delta^{4,6,22}$-ergostatrien-3-one.

(18) F. A. Askew, S. N. Farmer and G. A. R. Kon, *ibid.*, 1399 (1936).

of potassium hydroxide required nearly one hour, at which time the gas up-take corresponded to two moles. After one recrystallization, there was obtained 0.55 g. of $\Delta^{8(9)}$-22-isospirosten-3-one (VII) with m.p. 190–193°, $[\alpha]^{20}D$ −28°, no selective absorption in the ultraviolet. In the absence of potassium hydroxide, the yield was reduced to 0.4 g. Two recrystallizations from acetone afforded colorless crystals of the analytical sample with m.p. 200–202°, $[\alpha]^{20}D$ −30°, infrared carbonyl band at 1718 cm.$^{-1}$, yellow color with tetranitromethane.

Anal. Calcd. for $C_{27}H_{40}O_3$: C, 78.59; H, 9.77. Found: C, 78.98; H, 9.90.

$\Delta^{8(9)}$-22-Isospirosten-3α-ol (VIIIa).—The lithium aluminum hydride reduction of the ketone VII (1.2 g., m.p. 190–193°) was carried out exactly as described above and afforded 0.88 g. of alcohol with m.p. 209–213°. The analytical sample was obtained from ether with m.p. 211–213°, $[\alpha]^{20}D$ −3°, no precipitate with digitonin, yellow color with tetranitromethane, free hydroxyl but no carbonyl band in the infrared.

Anal. Calcd. for $C_{27}H_{42}O_3$: C, 78.21; H, 10.21. Found: C, 78.54; H, 10.49.

The **acetate VIIIb** was prepared in the usual manner and recrystallized from ether; m.p. 171–173°, $[\alpha]^{20}D$ ±0°. An ethereal solution of the acetate gave a black precipitate with osmium tetroxide in less than 30 minutes, typical[5] of $\Delta^{8(9)}$-stenols; the corresponding Δ^7-unsaturated allo derivative[19] required nearly a week for reaction with osmium tetroxide.

Anal. Calcd. for $C_{29}H_{44}O_4$: C, 76.27; H, 9.71. Found: C, 76.32; H, 9.81.

(19) G. Rosenkranz, J. Romo, E. Batres and C. Djerassi, *J. Org. Chem.*, **16**, 298 (1951).

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

Rutin Content of *Sophora japonica* L.

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Unopened flower buds of the Chinese Scholar tree are used for medicinal purposes in China under the name "Wai Fa," the "blossom of the tree." This material contains four to five times as much rutin as does buckwheat, our best domestic source of that glycoside. Specimens were collected from a tree near this Laboratory throughout the growing season. As the buds opened into flowers, the rutin content declined, and continued to decrease as the seed pods formed. When the seeds were in the soft green stage, the seed pods contained no rutin. Rutin in small quantities was isolated from the leaflets of the tree.

In 1853 Stein[2] isolated rutin from a commercial dyeing material known as "Chinese yellow berries" but of unknown botanical origin. He purified the pigment and identified it with "rutinic acid," discovered in garden rue by Weiss[3a] in 1842 and isolated from capers by Rochleder and Hlasiwetz.[3b] Martius[4] identified the "berries" as the unopened flower buds of *Sophora japonica* L., known in China as "Wai Fa" and used for centuries in Chinese medicine for hemorrhagic and other diseases much as rutin is now being used in western medicine. Common names for the tree are Japanese pagoda tree and Chinese Scholar tree.

Early supplies of rutin for pharmaceutical manufacturing were obtained from the leaves and blossoms of buckwheat.[5] Other sources, such as pansy petals, are much richer in rutin but not practicable. At first, sophora did not appear to be a promising material. None of it could be located in this country, and preliminary information from China indicated that no adequate supply could be expected from that quarter. Samples were obtained from Shanghai, through Dr. T. T. Pan, the analysis of which indicated a rutin content of 16.8% on a dry basis, that is, four to five times the rutin content of commercial buckwheat leaf meals. Evidently such a material would be of economic importance if sufficient could be obtained to satisfy manufacturing demands.

In 1947, a report was received that 10 tons of sophora had been imported into New York and was being offered for sale. A sample of this shipment contained 12.8% of rutin. The point of origin of this sophora was not revealed, but it may have been Hong Kong. Since then, shipments of sophora have arrived at intervals, and supplies are said to be plentiful. Samples of seven lots have been examined. They averaged 16.3% of rutin (dry basis), identified by chemical and spectrophotometric methods, with a maximum of 19.7 and a minimum of 12.8%. At present, sophora has displaced buckwheat as the source-material in rutin manufacture.

The sophora tree grows in northern China, being common around Peiping, in Korea and in parts of Japan. Japanese rutin manufacturers, however, import their sophora from China.[6] The tree is grown as an ornamental in the United States, being fairly common in some localities. Material for the present investigation was collected from a splendid specimen growing in the Morris Arboretum of the University of Pennsylvania, through the courtesy of Henry T. Skinner, Curator of the botanical garden.

Experimental

Flowering heads were collected at intervals throughout the growing season, beginning with the unopened flower buds and continuing as these developed into flowers and then formed seed pods. Part of the green material was immediately analyzed for rutin content. Other portions were used for moisture determination, or were dried either in air or at 110° in an electric oven.

Results of analyses of the green material are presented in Table I. The rapid drop in rutin content as the buds unfolded into bloom is consistent with similar behavior in buckwheat,[7] hydrangea[8] and pansies. The complete disappearance of rutin in the seed pods, whether young or

* Deceased.

(1) One of the laboratories of the Bureau of Agricultural and Industrial Chemistry, Agricultural Research Administration, United States Department of Agriculture. Article not copyrighted.

(2) W. Stein, *J. prakt. Chem.*, **58**, 399 (1853).

(3) (a) A. Weiss, *Pharm. Centr.*, **13** (II) 903 (1842); (b) Rochleder and Hlasiwetz, *Ann. Chem. Pharm.*, **82**, 197 (1852).

(4) Martius, *Arch. Pharm.*, **160**, 231 (1862).

(5) J. F. Couch, C. F. Krewson, J. Naghski and M. J. Copley, U. S. Dept. Agr., Bur. Agr. Ind. Chem., ERRL., AIC 115, 1946 (Processed).

(6) Tatsuo Keimatsu, Kyoto, personal communication.

(7) J. F. Couch, J. Naghski and C. F. Krewson, *Science*, **103**, 197 (1946).

(8) J. F. Couch and J. Naghski, THIS JOURNAL, **67**, 1419 (1945).