Raney nickels of different activity either returned crystalline V or resulted in complete decomposition.

THE ROLLIN H. STEVENS MEMORIAL LABORATORY
DETROIT INSTITUTE OF CANCER RESEARCH
DETROIT 1, MICH.

Steroidal Sapogenins. XLI. Willagenin, a New 12-Keto Sapogenin[1,2]

HAROLD E. KENNEY AND MONROE E. WALL

Received September 27, 1956

In the course of our investigations of the plant kingdom for steroidal sapogenins[3a,b,c] we have isolated a new sapogenin in low yield from *Yucca filifera*.[4] We wish to call this sapogenin willagenin.[5] This paper describes our investigations on the structure of willagenin.[6]

Extraction of the saponins from *Yucca filifera* sawdust[4] by our usual procedure[7] followed by acid hydrolysis gave a crude sapogenin mixture. Infrared analysis indicated that the chief constituent was sarsasapogenin and that there was also a minor ketonic impurity. Treatment of the sapogenin mixture with Girard's reagent T resulted in isolation of the ketonic fraction, willagenin, in pure form. Treatment with acetic anhydride gave a mono-acetate with a carbon and hydrogen analysis corresponding to $C_{29}H_{44}O_5$.

The infrared spectrum of willagenin acetate, shown in Fig. 1, gave valuable clues to the structure of this sapogenin. Thus the bands at 986(S), 919(S), 895(W) and 850 cm.$^{-1}$(M) showed that the sidechain was in the 22b (25L) series.[8,9] The split bands at 1252 cm.$^{-1}$ and 1234 cm.$^{-1}$ indicated that willagenin might have the ring A/B *cis* fusion in conjunction with a 3β-acetate.[10] The two strong bands at 1735 and 1708 cm.$^{-1}$ showed, respectively, a monoacetate and a ketone, possible at C_{12}.[11]

Wolff-Kishner reduction of willagenin gave sarsasapogenin (20α,22a,25L-spirostan-3β-ol), confirming the structural considerations deduced from the infrared data. All the structural features of willagenin were now clarified except for the location of the carbonyl group.

Since willagenin reacted with Girard's reagent T and could be reduced by the Huang-Minlon modification[12] of the Wolff-Kishner reduction, the carbonyl could not be C_{11}. The location of the infrared band at 1708 cm.$^{-1}$ narrowed the choice of carbonyl positions to C_1, C_2, C_4, C_6, C_7, and C_{12}.[13]

Using the method of molecular rotation differences[14] the contribution of the ketonic group in willagenin and its acetate was found to be +333 and +314, respectively.[15] The high positive values obtained strongly suggest that the location for the carbonyl group is C_{12}.[16] Thus, willagenin probably has the structure 3β-hydroxy-20α,22a,25L-spirostan-12-one.

The isolation of willagenin adds another link in the biogenetic pattern of steroidal sapogenins in the 5-n, 25L series. The other members in this series are the well known sarsasapogenin, found as a major component in many Yucca species[3a,b,c] and its recently discovered dihydroxy analog, markogenin.[17] The isomeric 5-n, 25D series includes smilagenin (3β-hydroxy), samogenin (2β, 3β-dihydroxy) and mexogenin (2β,3β-dihydroxy-12 ketone). The latter two were discovered in nature by Marker[18] and the location of the 2β-hydroxyl was clarified by Djerassi.[19] The last remaining member in this series (3β-hydroxy-12 ketone) has not

(1) Paper XL, "Simplified Procedure for the Qualitative Detection of Cardiac Glycosides," M. M. Krider, H. A. Monroe, M. E. Wall, and J. J. Willaman; presented at 130th National Meeting, AMERICAN CHEMICAL SOCIETY, Atlantic City, N. J., September 16–21, 1956.

(2) Article not copyrighted.

(3a) M. E. Wall *et al.*, *J. Am. Pharm. Assoc., Sci. Ed.*, **43**, 1 (1954); (b) *J. Am. Pharm. Assoc.*, **43**, 503 (1954); (c) *J. Am. Pharm. Chem. Assoc.*, **44**, 438 (1955).

(4) We wish to thank Mr. Rafael Rojas Gutierrez, Director General, Laboratorios Nacionales de Fomento Industrial, Mexico, D. F., for supplying us with plant material. Mr. Rojas informs us that the plant material used in our investigations came from a species identified as *Yucca filifera* Chabaud by Dr. Bassett Maguire, New York Botanical Gardens. *Y. filifera* grows at an altitude of 1000–1500 meters in Mexican arid zones. Dry stems from this species were sawed into chips. The sawdust obtained during the chipping process was the material sent to us for analysis by Mr. Rojas.

(5) In honor of Dr. J. J. Willaman, Head, Biochemical Section, Eastern Regional Research Laboratory, who has initiated and constantly encouraged plant chemical investigations at this laboratory.

(6) On account of the small quantity of pure material available many of the structural assignments were based on physical data.

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

(8) M. E. Wall, C. R. Eddy, M. L. McClennan, and M. E. Klumpp, *Anal. Chem.*, **24**, 1337 (1952).

(9) R. N. Jones, E. Katzenellenbogen, and K. Dobriner, *J. Am. Chem. Soc.* **75**, 158 (1953).

(10) R. N. Jones, P. Humphries, F. Herling, and K. Dobriner, *J. Am. Chem. Soc.*, **73**, 3215 (1951).

(11) R. N. Jones, P. Humphries, and K. Dobriner, *J. Am. Chem. Soc.*, **71**, 241 (1949); **72**, 956 (1950).

(12) Huang-Minlon, *J. Am. Chem. Soc.*, **71**, 3301 (1949).

(13) R. N. Jones and F. Herling, *J. Org. Chem.*, 19, 1252 (1954).

(14) D. H. R. Barton and W. Klyne, *Chemistry & Industry*, 755 (1948).

(15) M_D willagenin − M_D sarsasapogenin = +22 − (−312) = +333 M_D willagenin acetate − M_D sarsasapogenin acetate = −5 − (−319) − +314.

(16) Barton and Klyne, reference 14, give the following values for the molecular rotation contributions of ketones: C_1 = +67, C_2 = +98, C_4 = +25, all 5-allo series; C_6 (5n) = −224, C_{12} (5n + 5 allo) = +270.

(17) M. E. Wall, C. R. Eddy, S. Serota, and R. F. Mininger, *J. Am. Chem. Soc.* **75**, 4437 (1953).

(18) R. E. Marker *et al.*, *J. Am. Chem. Soc.*, **69**, 2167 (1947).

(19) C. Djerassi and J. Fishman, *J. Am. Chem. Soc.*, **77**, 4291 (1955).

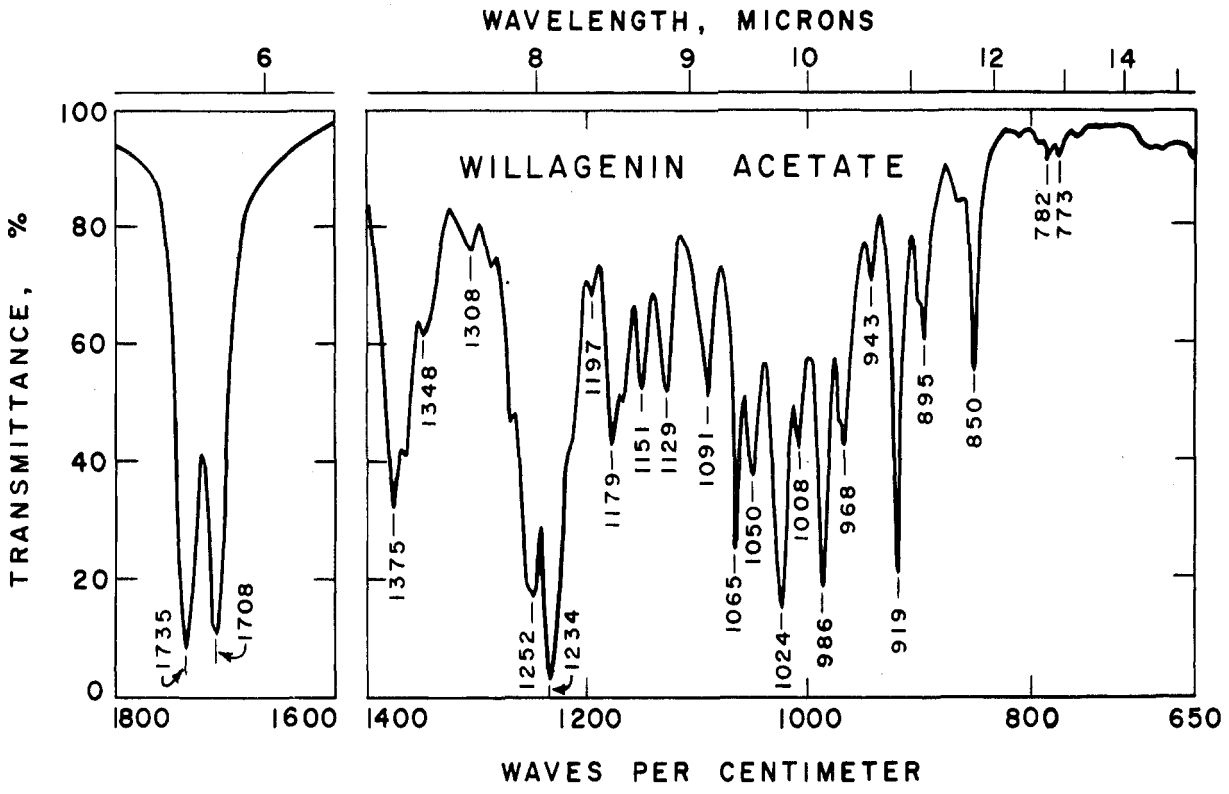

FIG. 1. WILLAGENIN ACETATE. (3β-hydroxy-20α, 22a, 25L-spirostan-12-one) 10.0 grams per liter in CS_2; 1.0-mm. cell.

been found in nature. Attempts to prepare it by hydrochloric acid isomerization of a minute quantity of willagenin were unsucessful.

EXPERIMENTAL

Melting points, optical rotation, ultraviolet and infrared spectra were determined in our usual manner.[20] *Yucca filifera* sawdust[4] (5.7 kg.) was extracted with ethanol and the saponin converted to sapogenin as described previously.[7] The crude sapogenin, 27.5 g., was dissolved in benzene and chromatographed on 300 g. of Florisil. Elution with benzene and chloroform gave semicrystalline fractions which contained some carbonyl (infrared assay). The benzene and chloroform eluates were combined and the solvent evaporated. The residue, 20.0 g., was treated with Girard's reagent T using the experimental conditions described by Mueller *et al.*[21] The ether soluble fraction was crystallized from methanol, 15.0 g., m.p. 196–198°; the infrared spectrum showed that the compound was sarsasapogenin.

Willagenin (3β-hydroxy-20α,22a,25L-spirostan-12-one). The water-soluble fraction from the Girard T separation described above was acidified to *p*H 1.0 with hydrochloric acid, heated 1 hr. on the steam bath and the flocculent precipitate collected, washed, and dried. Several crystallizations from methanol gave 0.5 g. of willagenin, flat rods, m.p. 166–168°, $[\alpha]_D^{25}$ +5.1°.

Willagenin acetate. Willagenin was treated with acetic anhydride–pyridine in the usual manner. After removing the solvent *in vacuo*, the residue was recrystallized three times from methanol, rods, m.p. 183–185°, $[\alpha]_D^{25}$ −1.0°; infrared spectrum is shown in Fig. 1.

Anal. Calcd. for $C_{29}H_{44}O_5$: C, 73.69; H, 9.38. Found: C, 73.90; H, 9.44.

Wolff-Kishner reduction of willagenin. Willagenin (100 mg.) was treated with hydrazine hydrate and alkali in a mixture of ethanol–ethylene glycol using the experimental conditions described by Huang-Minlon.[12] After the usual ether work-up, the residue was crystallized from methanol to yield 70 mg., m.p. 195–198°, infrared spectrum identical with that of sarsasapogenin.

EASTERN REGIONAL LABORATORY
EASTERN UTILIZATION RESEARCH AND DEVELOPMENT DIVISION
AGRICULTURAL RESEARCH SERVICE
U. S. DEPARTMENT OF AGRICULTURE
PHILADELPHIA 18, PA.

(20) M. E. Wall, S. Serota, and C. Roland Eddy, *J. Am. Chem. Soc.*, **77**, 1234 (1955).

(21) G. P. Mueller, R. E. Stobaugh, and R. S. Winniford, *J. Am. Chem. Soc.*, **75**, 4888 (1953).

Optical Activity of Phytol

NICKY BEREDJICK AND CONRAD SCHUERCH

Received October 8, 1956

Phytol was first isolated as a constituent of the chlorophyll molecule by R. Willstätter and Ferdinand Hocheder,[1] who reported an optical activity of $[\alpha]_D^{20}$ +0.79° for crude phytol. Subsequent to distillation in vacuum, the optical activity could not be detected.

F. G. Fisher[2] and K. Lowenberg[3] proved the

(1) R. Willstätter and Ferdinand Hocheder, *Ann.*, **354**, 205 (1907).

(2) F. G. Fisher, *Ann.* **464**, 69 (1928).

(3) F. G. Fisher and K. Lowenberg; *Ann.* **475**, 183 (1929).