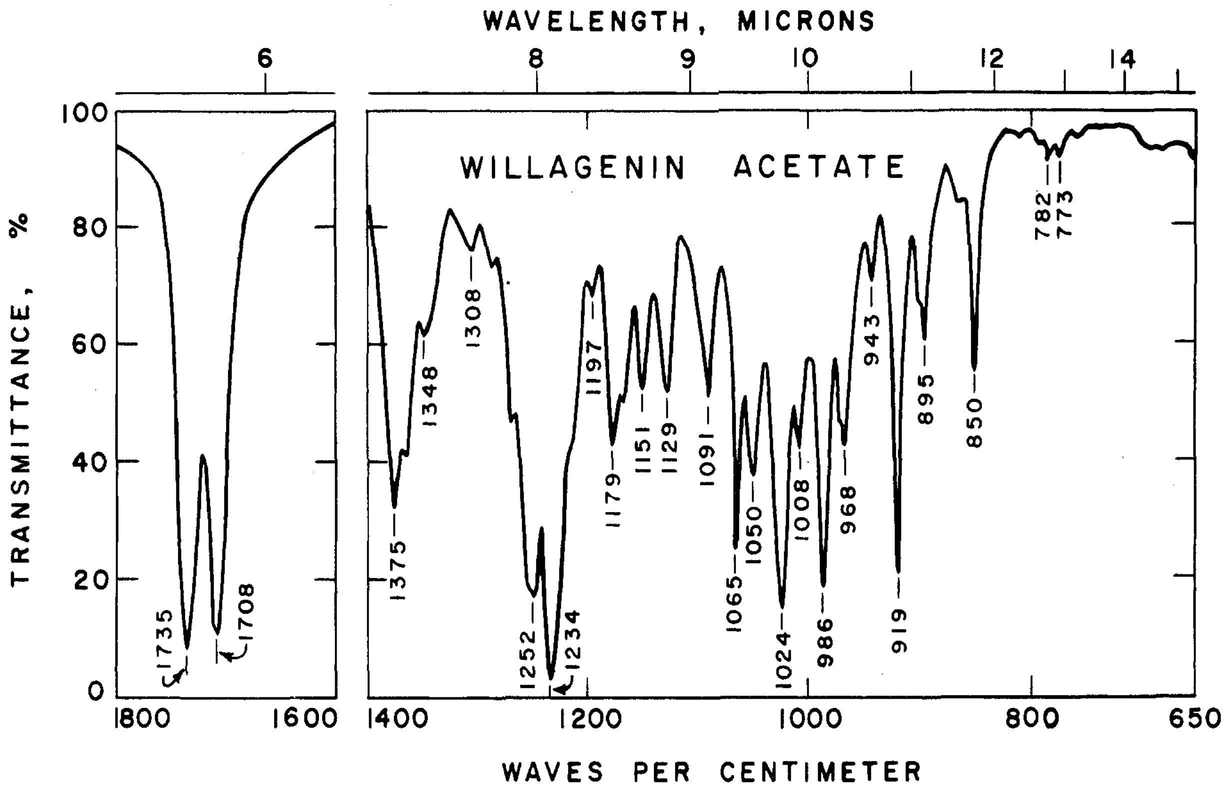

FIG. 1. WILLAGENIN ACETATE. (3β-hydroxy-20α, 22a, 25L-spirostan-12-one) 10.0 grams per liter in CS_2; 1.0-mm. cell.

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.

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

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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.

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Optical Activity of Phytol

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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).

structure of phytol and reported a successful synthesis, but were unable to observe any optical activity in either crude or distilled phytol.

T. Wagner-Jauregg[4] observed a small rotation on crude phytol (α +0.08°, $[\alpha]_D$ +0.79°), and no optical activity on distilled fractions. He concluded that native phytol is either racemic or had a very small optical rotation.

Samples of native phytol examined by P. Karrer *et al.* showed very small to zero rotations. The same workers synthesized an optically active levorotatory phytol (not an enantiomorph of native (+)-phytol, but a mixture of two diastereoisomers), which had a small rotation, α −0.18°, $[\alpha]_D^{18}$ −0.20°.[5,6]

A persisting four degree difference in the melting points of the allophanates of *d,l*-α-tocopherol, whether prepared from native or semisynthetic phytol, had been found. It was suggested that this might be due to the different steric nature of native and semisynthetic phytols.[7]

Both the native and semisynthetic phytols gave on ozonolysis 2,6,10,trimethylpentadecanone-14 (I) and on oxidation 4,8,12,trimethyltridecanoic acid (II).

$$(CH_3)_2CH(CH_2)_3\overset{CH_3}{\overset{|}{C}}H(CH_2)_3\overset{CH_3}{\overset{|}{C}}H(CH_2)_3\text{—}\overset{CH_3}{\overset{|}{C}}{=}O$$

I ($a_{1,2}$ ±0.22°)

$$(CH_3)_2CH(CH_2)_3\overset{CH_3}{\overset{|}{C}}H(CH_2)_3\overset{CH_3}{\overset{|}{C}}HCH_2CH_2COOH$$

II ($a_{1,2}$ ±0.24°)

The preparation of both products results in an increase in optical rotation.

Both the very weakly active and the seemingly inactive samples of native phytol as well as the semisynthetic phytol, however, gave phytadienes (III) which had distinct optical rotations.

$$(CH_3)_2CH(CH_2)_3\overset{CH_3}{\overset{|}{C}}H(CH_2)_3\overset{CH_3}{\overset{|}{C}}H(CH_2)_2CH = \overset{CH_3}{\overset{|}{C}}CH{=}CH_2$$

III	Native phytol	a = 0 to +0.17°
(+)	Phytadiene	a = +0.89°
	Synthetic (−) phytol	a = −0.18°
(−)	Phytadiene	a = −0.25°

Consequently, phytol was termed a latently optically active substance. It was assumed that phytol preparations might be accompanied either by small amounts of levorotatory impurity, which would obscure the small dextrorotation of the phytol itself or by a negligible amount of dextrorotatory substance to which the observed rotation should be ascribed.

It is our intention to offer an explanation for the small or immeasurable optical rotation of phytol. This problem seems to be closely related to the problem of configuration and optical activity of vinyl polymers.[8,9] Phytol can be described as a low molecular polymer (tetramer) of three isopentane units and one modified isopentane unit.

$$CH_3\overset{CH_3}{\overset{|}{C}}HCH_2CH_2\text{——}CH_2\overset{CH_3}{\overset{|}{C}}HCH_2CH_2\text{——}$$

(6)

$$CH_2\overset{CH_3}{\overset{|}{C}}HCH_2CH_2\text{——}CH_2\overset{CH_3}{\overset{|}{C}}{=}CHCH_2OH$$

(10)

Work on simple systems and small molecules[10] indicates that any structural dissimilarity more than a few atoms away from the asymmetric center will result in little contribution to the optical activity of the center. Therefore, contributions of chain length differences and end groups to the optical activity were expected[8,9] and appeared[11] to be negligible.

The C_6 and C_{10} atoms in the phytol molecule are asymmetric centers. The point of structural dissimilarity relevant to the C_6 center is removed, however, to the fifth carbon atom whereas the structural dissimilarity relevant to the C_{10} center is removed to the fourth carbon atom. Therefore only a small optical activity is observed. Any modification in the molecule which would "move" the points of structural dissimilarity closer to the asymmetric center will be accompanied by an increase in optical activity. This is the case in 4,8,12-trimethyltridecanoic acid (II). The point of structural dissimilarity relevant to the C_{10} asymmetric center is now "moved" to the third carbon atom and the angular rotation has increased from +0.17 to +0.24° (an increase of 41%).

In the case of (+)-phytadiene the point of structural dissimilarity revelant to the C_{10} asymmetric center is now "moved" to the third carbon atom. A shift of the absorption band to longer wave lengths has also occurred.[7] The angular rotation has increased from 0° and +0.17° to +0.89°, the molar rotations show a fivefold increase.

$$[M]_{\text{phytol}} = +0.58°,\ [M]_{\text{phytadiene}} = +3.0°$$

The smaller increase in the angular rotation in the case of (+)-phytadiene as obtained from semisynthetic (−)-phytol is due to the fact that the latter is a mixture of two diastereoisomers.

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Synthesis of *p*-Biphenylyl β-D-Glucopyranosiduronic Acid and Its Optical and Crystallographic Properties

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A compound believed to be *p*-biphenylyl β-D-glucopyranosiduronic acid has been isolated as a urinary metabolite of *p*-phenylphenol in rabbits[1] and of biphenyl in rats.[2] In order to establish the structure, authentic *p*-biphenylyl β-D-glucopyranosiduronic acid was synthesized by chemical means and shown to be the same as the metabolite obtained by workers at this laboratory from the urine of rats on a diet containing biphenyl.[3] This work was conducted in connection with chronic-toxicity studies on biphenyl.

EXPERIMENTAL

p-Biphenylyl β-D-glucopyranosiduronic acid. A modification of the method used by Marsh[4] to oxidize a glucopyranoside to a glucopyranosiduronic acid was employed. Platinum on charcoal was used as a catalyst instead of platinum black. A suspension of 1.85 g. of *p*-biphenylyl β-D-glucopyranoside[5] and 0.400 g. of platinum on charcoal in 300 ml. of water was heated with stirring to 65°C., while oxygen was bubbled through the mixture over a period of 4 hr. A solution of sodium bicarbonate was added when necessary to keep the reaction neutral to litmus. After 4 hr. reaction the mixture was filtered and the insoluble cake of unreacted crystalline glucoside and catalyst was resuspended in water, heated to 65°C., and allowed to react as before. The isolation was repeated and a fresh portion of platinum on charcoal catalyst (0.200 g.) was added for the third oxidation treatment. The filtrates were combined and concentrated under reduced pressure. The *p*H was adjusted to 3 with dilute hydrochloric acid. The precipitated material was filtered and a yield of 0.330 g. (23% based on reacted starting material) of needle-like crystals was obtained from hot water. In addition 0.54 g. of starting material was recovered. The analyses of the product remained unchanged upon repeated crystallization.

Two dimensional paper chromatography was conducted on the synthetic material and the metabolite from ra urine. These materials chromatographed alike and both showed the same degree and kind of fluorescence. Upon acid hydrolysis the synthetic glucuronide showed the presence of only *p*-phenylphenol and glucuronic acid by paper chromatography. The analyses of the synthetic material are as follows:

Anal. Calcd. for $C_{18}H_{18}O_7$: C, 62.4; H, 5.2. Found: C, 62.0; H, 5.31 $[\alpha]_D^{25}$ (*c* 1, 95% ethanol) −80.2;[6] melted with decomposition, 185–187°C. $[\alpha]_D^{28}$ (*c* 1, ethanol) −85.2;[2] m.p., 185°.[2] The rotation given by Dodgson,[1] $[\alpha]_D^{20}$ −90.6, was measured from a 0.1*N* sodium hydroxide solution.

Optical and crystallographic properties of synthetic p-biphenylyl β-D-glucopyranosiduronic acid. Form and habit. Crystals grown by slowly cooling a hot aqueous solution are colorless in splintery clusters of nearly parallel needles or blades tapering to slender points. Longitudinal striations are common. Transverse cleavage is likely to occur when crystals are handled or when the cover glass is moved on crystals immersed in oil. Common views of the crystals all show parallel extinction with the slow ray crosswise, α lengthwise. The crystal system is probably orthorhombic.

Refractive indices. (Sodium light, 27°C.) $\alpha = 1.558$, $\beta = 1.602$, $\gamma = 1.73$.

Optic axial angle. (+) $2V = 64°40'$ calculated from α, β, γ. $2E = 118°$ calculated from α, β, γ.

Dispersion. $(r > v)$ slight.

Optic orientation. The axial plane is lengthwise, α is lengthwise. Centered Bx_a interference figures are obtainable from some blades, although the angle 2*E* is too large to measure accurately (melatopes at edge of field). Some crystals appear to be twisted so that the transverse index changes from β to γ along the length of the crystal. β and γ were determined on crystals whose orientation was checked by means of interference figures.

The optical and crystallographic properties of the metabolite from rat urine were the same as those of the synthetic *p*-biphenylyl β-D-glucopyranosiduronic acid.

X-ray powder diffraction. The *d* values for the major lines and their relative intensities are reported in Table I.

TABLE I

X-RAY POWDER DIFFRACTION DATA[a]

d, A	I/I_1	*d*, A	I/I_1	*d*, A	I/I_1
9.46	41.7	4.13	46.7	3.18	16.7
8.65	25.0	4.01	83.3	3.08	20.8
6.68	37.5	3.85	40.0	2.93	14.6
6.12	16.7	3.75	8.3	2.84	4.2
4.84	100.0	3.67	15.0	2.76	7.3
4.58	53.3	3.49	37.5	2.66	16.7
4.40	16.7	3.37	29.2	2.42	12.5
4.21	83.3	3.28	10.4	1.878	8.3

[a] The camera radius was 7.181 cm. λ for CuKα = 1.5418 A. and a nickel filter was used. The relative intensities were visually determined with a calibrated intensity scale.

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