litmus, the solution was acidified with one drop of acetic acid, and a solution of 1.4 ml. of phenylhydrazine in 3 ml. of 25% acetic acid was added. The D-manno-L-*manno*-octose phenylhydrazone which crystallized was collected by filtration, washed with water, ethanol and then ether. The yield was 3.5 g. (84.5%) of product melting at 207–209°.

Conversion of D-Manno-L-*manno*-octose Phenylhydrazone into D-Manno-L-*manno*-octose.—D-Manno-L-*manno*-octose phenylhydrazone (7.5 g.) was refluxed for 2.5 hours with 76 ml. of water, 7.6 ml. of benzaldehyde, 0.76 g. of benzoic acid and 15 ml. of ethanol. After cooling, the solution was decanted from benzaldehyde phenylhydrazone, the decantate extracted thrice with chloroform, and the aqueous layer concentrated *in vacuo* to a sirup which crystallized slowly after the addition of glacial acetic acid and seeding. The yield of octose, m.p. 150–152°, $[\alpha]^{20}D$ −7.4° (equilibrium) in water (*c* 3) amounted to 3.6 g. (66%).

Direct Preparation of D-Manno-L-*manno*-octose Phenylhydrazone and D-Manno-L-*manno*-octose Phenylosazone from D-Manno-D-*gala*-heptose and Nitromethane—Ten grams of heptose was shaken for 48 hours with nitromethane, sodium methoxide and methanol as described above. The sodium salts of the C-nitroalcohols were dissolved in 48 ml. of water, and added with stirring to a cooled mixture of 10 ml. of sulfuric acid and 12 ml. of water. After neutralization to congo red with sodium carbonate monohydrate and to litmus with sodium bicarbonate, the solution was acidified with acetic acid and a solution of 5 ml. of phenylhydrazine in 10 ml. of 25% acetic acid was added. The D-manno-L-*manno*-octose phenylhydrazone which crystallized was collected by filtration, washed with water, ethanol and ether; 3.6 g. (23%), m.p. 206–209°. In a subsequent larger run a 29% yield of phenylhydrazone was obtained.

To the mother liquor was added 8.5 ml. of phenylhydrazine and 6 ml. of glacial acetic acid and after heating on the steam-bath for three hours, the phenylosazone began to precipitate. After filtration and washing with ethanol and ether, the yield of D-manno-L-*manno*-octose phenylosazone amounted to 2.0 g. (10%).

BETHESDA, MD.

[CONTRIBUTION FROM THE DEPARTMENT OF BIOCHEMISTRY, UNIVERSITY OF WISCONSIN]

Isolation of Δ^7-Stigmastenol from Wheat[1]

BY D. R. IDLER,[2] A. A. KANDUTSCH[3] AND C. A. BAUMANN

RECEIVED FEBRUARY 23, 1953

Δ^7-Stigmastenol has been isolated from the sterols of wheat germ oil by chromatography of the azoyl esters followed by crystallization. The sterol comprises 3% of the total sterols in this source. It appears to be one of the components of "α_3-sitosterol."

Δ^7-Sterols could be useful as starting materials for the introduction of the biologically important 11-keto group,[4,5] but the known examples are not abundant in sources that are readily available.[6–8] In the present study the azoyl esters of wheat sterols were separated into three zones by chromatography, and Δ^7-stigmastenol (Δ^7-spinastenol)[9] was isolated as the less soluble component of the middle zone. It comprised 3% of the original sterols. The upper zone sterols (8% of the total) appeared to be a mixture of other Δ^7-sterols. Thus wheat is at least as rich as oats[10] in this type of compound.

Derivatives of Δ^7-stigmastenol showed similarities in melting point and rotation to derivatives of α_3-sitosterol (Table I), an uncharacterized sterol isolated from wheat by crystallization,[11] and most of the infrared spectra of the two preparations were also similar. A significant difference, however, was a lack of absorption at 12.22 μ in the spectrum of Δ^7-stigmastenol, whereas α_3-sitosterol showed a moderately strong band in this region. Furthermore, the band at 11.25 μ was considerably weaker than the 11.46 μ band in the spectrum of Δ^7-stigmastenol, whereas the intensities were nearly equal in the spectrum of α_3-sitosterol (Fig. 1). In this connection the spectrum of the upper zone sterol is

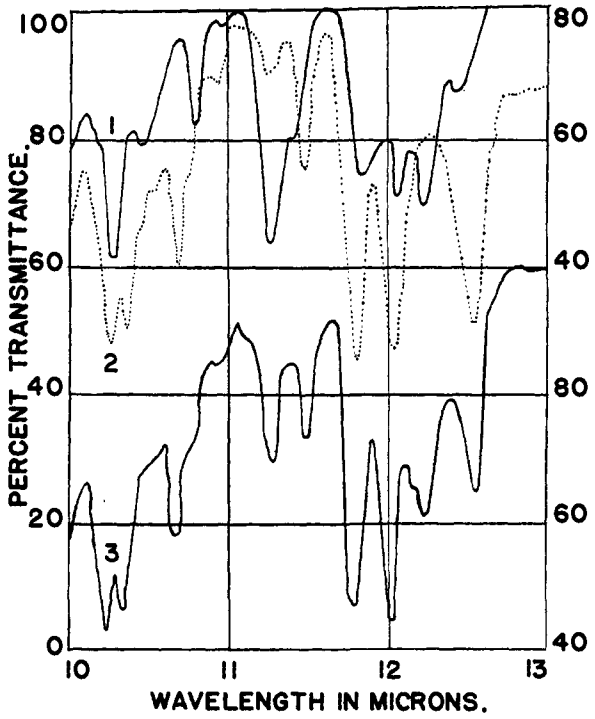

Fig. 1.—Comparison of the infrared spectra of the upper chromatographic zone of wheat sterols 1 (scale upper right), Δ^7-stigmastenol 2 (dotted line, scale on the left) and α_3-sitosterol 3 (scale lower right).

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(2) Babcock fellow, 1952–1953.

(3) Public Health research fellow of the National Cancer Institute, 1953–1954.

(4) G. Stork, J. Romo, G. Rosenkranz and C. Djerassi, THIS JOURNAL, **73**, 3546 (1951).

(5) L. F. Fieser and J. E. Herz, *ibid.*, **75**, 121 (1953).

(6) L. F. Fieser and M. Fieser, "Natural Products Related to Phenanthrene," 3rd Ed., Reinhold Publ. Cop., New York, N. Y., 1949, pp. 282–283, 294–297.

(7) L. F. Fieser, THIS JOURNAL, **73**, 5007 (1951).

(8) D. R. Idler and C. A. Baumann, *J. Biol. Chem.*, **195**, 623 (1952).

(9) In the present discussion the product from wheat is referred to as "Δ^7-stigmastenol" and the product prepared from α-spinasterol as "Δ^7-spinastenol."

(10) D. R. Idler, S. W. Nicksic, D. R. Johnson, V. W. Meloche, H. A. Schuette and C. A. Baumann, THIS JOURNAL, **75**, 1712 (1953).

(11) S. Bernstein and E. S. Wallis, *ibid.*, **61**, 1903 (1939).

of interest, since it contained strong bands at both 11.25 and 12.3 μ. Thus, the spectrum of α_3-sitosterol resembled that to be expected of a mixture of Δ^7-stigmastenol and the upper zone sterol. The molecular rotation of α_3-sitosterol was less than that of Δ^7-stigmastenol, and the rotational differences between the esters and the free sterol were more positive (Table I). Barton[12] was unable to assign the unsaturation of α_3-sitosterol on the basis of molecular rotational differences.

TABLE I

SIMILARITIES BETWEEN α_3-SITOSTEROL, Δ^7-SPINASTENOL AND Δ^7-STIGMASTENOL FROM WHEAT

	α_3-Sitosterol[11] (wheat)		Δ^7-Stigmastenol (wheat)		Δ^7-Spinastenol[17] (from α-spinasterol)	
	M.p., °C.	α_D	M.p., °C.	α_D	M.p., °C.	α_D
Free sterol	142–143	+ 5.2	146	+ 9.1	144–145 (146)[a]	+11
Acetate	152–153	+ 6.1	159	+ 6.6	156–157 (159)[a]	+ 8 (+ 7.7)[a]
Benzoate	173–175	+12.0	181	+12.0	180.5 (181)[a]	+13
Azoate	.....	....	214	....	.....	.....

[a] Our preparation.

Experimental[13]

Colorimetric determinations of the sterols were carried out as follows: 50–200 γ of the sterol in 2 ml. of acetic acid was treated with 4.2 ml. of a previously chilled 20:1 mixture of acetic anhydride:sulfuric acid. The reaction mixture was kept at 24.5° and readings were taken at appropriate intervals.[14]

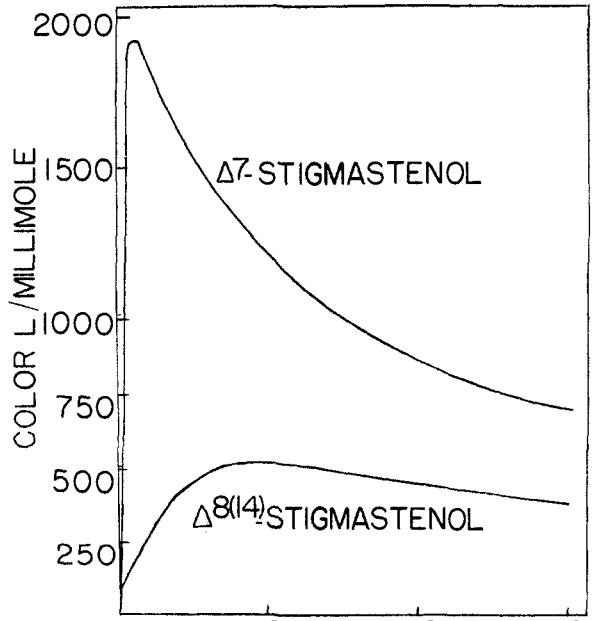


Fig. 2.—Reactivity of the Δ^7-stigmastenol from wheat with the modified Liebermann–Burchard reaction.

Preparation of Crude Sterols.—Five quarts of wheat germ oil were saponified with alcoholic KOH under nitrogen. The solid unsaponifiable matter was crystallized twice from Skellysolve-B without chilling and once from methanol, yield 40 g. of mixed sitosterols, m.p. 139–140°.

Chromatography.—Azoyl esters of the sterols were chromatographed in 3-g. batches on a Zechmeister column, 10.6 cm. inside diameter, by the procedure described for oat sterols.[8,10] Three sterol zones developed after 20 hours. The upper zone ester (8–10%) was separated from the middle zone ester (10–12%) by 1 cm. and this from the lower zone ester (76–80%) by 2 cm. The separated zones were extruded from the column and eluted with a benzene:ether:ethanol mixture.

Isolation of Δ^7-Stigmastenyl Azoate.—In a typical recrystallization 372 mg. of the middle zone azoyl esters was crystallized from 125 ml. of benzene:ethanol (1:2) and yielded 188 mg., m.p. 188°—?. A second crystallization out of 50 ml. yielded 108 mg. of ester, m.p. 204.5°, and this product gave 85 mg., m.p. 214°, out of 50 ml. Ten further crystallizations gave 20 mg. of ester, m.p. 214°.

Anal. Calcd. for $C_{42}H_{58}O_2N_2$: C, 80.98; H, 9.38. Found: C, 81.09; H, 9.52.

Δ^7-Stigmastenol.—Hydrolysis yielded long needles on crystallization from methanol, m.p. 146°. The sterol contained 2.5% of a $\Delta^{5,7}$-sterol as measured by the ultraviolet spectrum; no maxima were found for the sterol at the normal concentration used to detect a diene system. The sterol was converted to the acetate and dissolved in 20 volumes of xylene containing 15 parts of maleic anhydride to 1 part of the 5,7-dienol contaminant. The solution was refluxed for 16 hours, saponified and worked up in the usual manner.[15] The Δ^7-stigmastenol now showed no ultraviolet absorption, m.p. 146°, $[\alpha]^{25}D$ +9.1°. There was no depression on mixed melting with a preparation of Δ^7-spinastenol prepared by the hydrogenation of α-spinasterol. The sterol was precipitated by digitonin.

Anal. Calcd. for $C_{29}H_{50}O$: C, 83.99; H, 12.15. Found: C, 83.97; H, 11.94.

Derivatives.—The acetate crystallized from ethanol in plates, m.p. 159°, $[\alpha]^{26}D$ +6.6°.

Anal. Calcd. for $C_{31}H_{52}O_2$: C, 81.58; H, 11.48. Found: C, 80.95; H, 11.41.

The benzoate crystallized from acetone in plates, m.p. 181°, $[\alpha]^{26}D$ +12°. The melting points of the derivatives were undepressed on admixture with authentic Δ^7-spinastenol derivatives. Molecular rotational differences: Δ^7-spinastenol; Δ^1 −12, Δ^2 +8. Found: Δ^1 −10, Δ^2 +12.

Hydrogenation of α-Spinasteryl Acetate.—Ninety mg. of the acetate, m.p. 188°, in purified ethyl acetate[16] was hydrogenated overnight with Adams platinum oxide catalyst. Δ^7-Spinastenyl acetate, m.p. 159°, crystallized from ethanol in 95% yield. Barton has reported a yield of only 22% after 6 hours of hydrogenation.[17]

Hydrogenation of Δ^7-Stigmastenyl Acetate.—There was no uptake of hydrogen when 30 mg. of the acetate (m.p. 159°) was shaken with 25 ml. of Adams catalyst in glacial acetic acid. The reduction product crystallized in plates, m.p. 115°. Pure $\Delta^{8(14)}$-spinastenyl acetate did not depress the melting point. Alkaline hydrolysis of both acetates yielded sterols that crystallized from methanol in plates, m.p. 115°; mixed m.p. 115°. The infrared spectrum of the $\Delta^{8(14)}$-wheat sterol was identical to that of $\Delta^{8(14)}$-spinastenol prepared from α-spinasterol; the two products also showed a similar response to the Liebermann–Burchard reagent (Fig. 2).

Perbenzoic Acid Titration.—On standing for five days at −5° in an excess of perbenzoic acid in $CHCl_3$, 15 mg. of Δ^7-stigmastenyl acetate from wheat took up 1.04 mg. of oxygen; theory for two atoms of oxygen, 1.05 mg. The Δ^7-bond consumes two atoms of oxygen under these conditions.[10]

Other Properties.—On treatment with the modified Liebermann–Burchard reagent the sterol reacted at a rate[14,18]

(12) D. H. R. Barton, *J. Chem. Soc.*, 813 (1945).

(13) Melting points are corrected. All solutions for rotations were prepared in 2.5 ml. of chloroform solution, and the measurements made with a Schmidt and Haensch polarimeter No. 52-b with monochromator. Ultraviolet absorption spectra were taken of ethanol solutions. The carbon and hydrogen analyses were made by the Micro-Tech Laboratories, Skokie, Illinois. We are indebted to D. R. Johnson, R. Meiklejohn and V. W. Meloche of the Chemistry Department of this University for the infrared spectra.

(14) D. R. Idler and C. A. Baumann, *J. Biol. Chem.*, July, (1953).

(15) A. Windaus and A. Lüttringhaus, *Ber.*, **64**, 850 (1931).

(16) L. F. Fieser, "Experiments in Organic Chemistry," D. C. Heath and Co., New York, N. Y., 1941, p. 364.

(17) D. H. R. Barton, *J. Chem. Soc.*, 1356 (1948).

(18) P. R. Moore and C. A. Baumann, *J. Biol. Chem.*, **195**, 615 (1952).

characteristic of Δ^7-sterols (Fig. 2), and as expected[14] the chromophore was somewhat less intense than that of Δ^7-cholestenol. The infrared spectrum was identical to that of a sample of Δ^7-spinastenol prepared from α-spinasterol, and was also very similar to that of Δ^7-cholestenol.[19]

(19) D. R. Johnson, D. R. Idler, V. W. Meloche and C. A. Baumann, THIS JOURNAL, **75**, 52 (1953).

Acknowledgment.—We are indebted to Dr. O. Wintersteiner of the Squibb Company for a generous sample of α-spinasteryl acetate and to Dr. E. S. Wallis, Princeton University, for samples of α_3-sitosteryl acetate and benzoate.

MADISON, WISCONSIN

[CONTRIBUTION FROM THE DEPARTMENT OF BIOCHEMISTRY OF THE SOUTHWESTERN MEDICAL SCHOOL]

16-Substituted Steroids. VIII. 1,3,5(10)-Estratrien-3-ol-16-one

BY MAX N. HUFFMAN[1] AND MARY HARRIET LOTT[1]

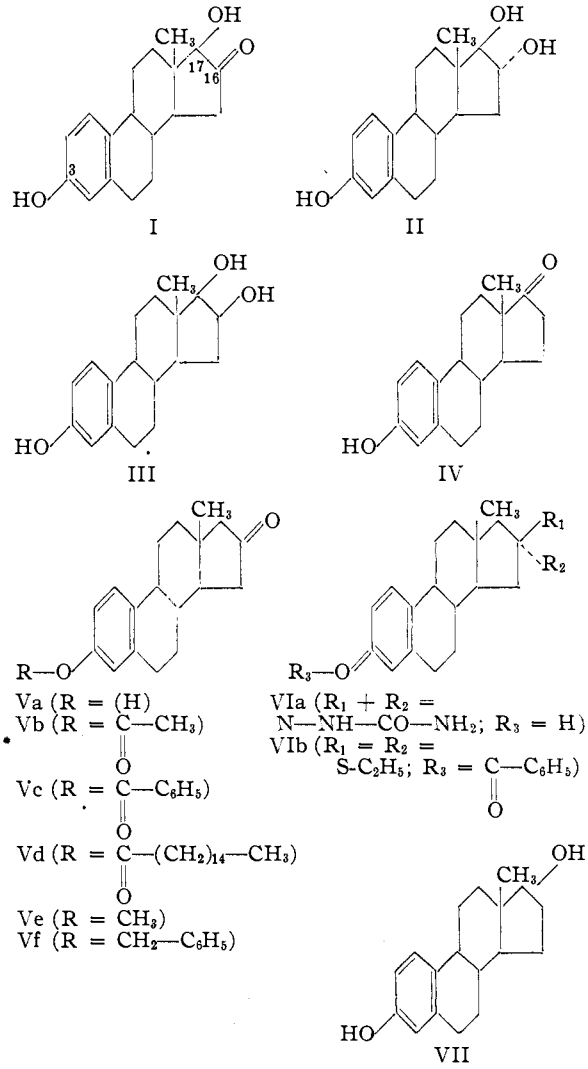
RECEIVED MARCH 19, 1953

The mild Clemmensen reduction of 1,3,5(10)-estratrien-3,17β-diol-16-one produces a mixture of estrone and 1,3,5(10)-estratrien-3-ol-16-one. The latter was characterized as the semicarbazone, acetate, benzoate, palmitate, methyl ether and benzyl ether. The shifting of the carbonyl from C_{17} to C_{16} on the naturally-occurring estratrien nucleus resulted in a powerful rotatory effect in the negative direction. The mechanism by which 1,3,5(10)-estratrien-3-ol-16-one is formed in the Clemmensen reduction of 1,3,5(10)-estratrien-3,17β-diol-16-one is discussed.

In 1943, 1,3,5(10)-estratrien-3,17β-diol-16-one[2,3] (I) was submitted to reduction with amalgamated zinc in aqueous ethanolic hydrochloric acid in the hope of obtaining one of the four possible 16,17-epimers of estriol (II). There was obtained instead a material which was soluble in 0.5 *N* sodium hydroxide, and which gave both a monobenzoate and a semicarbazone whose analyses agreed unexpectedly with those of a parent $C_{18}H_{22}O_2$ compound.

The mild Clemmensen reduction of 1,3,5(10)-estratrien-3,17β-diol-16-one was repeated several times, always with the finding that a ketonic material of varying melting point was obtained whose analysis was correct for estrone (IV). One such preparation, under assay, contained 50% estrone; another such preparation, after further reduction of carbonyl to carbinol, furnished pure estradiol-3,17β (VII).

It finally became clear that our reduction of 1,3,5(10)-estratrien-3,17β-diol-16-one[4,5] produced a varying mixture of estrone and an isomer of estrone. We were eventually successful in obtaining pure the new $C_{18}H_{22}O_2$ steroid, which showed an optical rotation of $[\alpha]^{25}D$ $-87°$ (95% ethanol). That this new compound possesses the unaltered, naturally-occurring estratrien nucleus was established by hydrogenolysis[4] of its 3-benzoxy-16-diethyl thioketal to desoxoestrone benzoate (followed by saponification to desoxoestrone) as shown by mixed melting point comparison using authentic desoxoestrone benzoate (and using authentic desoxoestrone). Inasmuch as the new 1,3,5(10)-estratrien-3-ol-monoketone was formed from the reduction of a parent molecule which bears oxygen functions at C_{16} and C_{17}, the only logical structural formulation for it is



that of a 1,3,5(10)-estratrien-3-ol-16-one (Va), for it cannot bear the carbonyl at C_{17}.

The optical rotation value for 1,3,5(10)-estratrien-3-ol-16-one ($-87°$) is interestingly some 250° more negative than that of naturally-occurring estrone

(1) Oklahoma Medical Research Foundation, Oklahoma City' Oklahoma.

(2) M. N. Huffman, THIS JOURNAL, **64**, 2235 (1942).

(3) M. N. Huffman and M. H. Lott, *J. Biol. Chem.*, **172**, 325 (1948).

(4) M. N. Huffman and M. H. Lott, THIS JOURNAL, **71**, 719 (1949).

(5) In this manuscript we are using the presently accepted terminology for the stereo configuration at C_{17} of the steroid nucleus. Structural determinations in the field of steroidal 16,17-ketols and 16,17-glycols previously presented by us[4] were based upon the general belief then held that the C_{17}-carbinol of testosterone and estradiol possessed the α-configuration.