THE SYNTHESIS AND BIOLOGICAL ACTIVITY OF STEROIDAL ESTERS OF 3-INDOLEACETIC ACID

Thesis for the Degree of M. S.

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John Frederick Hofert

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THE SYNTHESIS AND BIOLOGICAL ACTIVITY OF STEROIDAL ESTERS OF 3-INDOLEACETIC ACID

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AN ABSTRACT

Submitted to the College of Agriculture
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Department of Agricultural Chemistry

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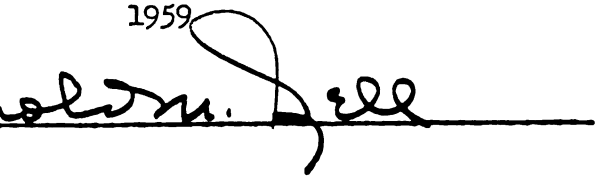
Approved 

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ABSTRACT

Recent reports in the literature indicate that plant growth can be effected by lipoidal substances. In the past, non-polar derivatives of 3-indoleacetic acid, such as alkyl esters, have been tested in various bioassays yielding the interesting results that some are more active than free 3-indoleacetic acid.

Five steroidal esters of 3-indoleacetic acid have been prepared. The syntheses were accomplished for the cholesteryl, 7-dehydrocholesteryl, and ergosteryl esters by way of the acid chloride, free sterol, and silver carbonate in benzene at room temperature. Cholestanyl and β-sitosteryl esters were prepared using the acid chloride, sterol, and silver carbonate in refluxing petroleum ether (40° - 45° C.). Physical constants (i.e., melting point, optical rotation and ultraviolet spectra) were reported for the new compounds.

Ergosterol, β-sitosterol, and the 3-indoleacetic acid esters were assayed using the induction of parthenocarpic fruit in the tomato. The two free sterols, cholesteryl, cholestanyl and β-sitosteryl esters were inactive, while 7-dehydrocholesteryl and ergosteryl esters showed slight activity, but much less than the parent acid.

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TABLE OF CONTENTS

LIST OF TABLES

INTRODUCTION

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Lipoid-Growth Regulator Relations

3-Indoleacetic acid (IAA) is now a well established native plant growth substance. Detection by chromatographic techniques has been reported in at least 45 different tissues with wide ontogenetic variation (1). However isolation of 3-indolyacetonitrile by Henbest (2) showed that IAA was not unique among the indolic plant growth regulators.

The role which IAA plays in the growth panorama is not well established. Bonner et al. (3) feel that growth induced by auxins is the result of increased water uptake, and may be influenced by a primary effect on the cell wall. This action on the cell membrane may well be on the pectic substances (1). For example, pectin methyl esterase has been shown to increase when growth is stimulated by IAA (1).

It has become increasingly clear throughout the past few years that indolic substances are not the only plant growth regulators. Isolation of a few milligrams of a growth promoter from 3 tons of Maryland Mommouth tobacco has been reported, which from I.R. spectral data seems to be a fatty alcohol in the range C_{18} to C_{27} (4). Noteworthy was the fact that no indolic compounds could be detected.

During a bioassay using pea epicotyls, growth is not as great as in the intact plant, even in the presence of optimum concentrations of IAA, gibberellic acid (GA_3), sucrose and cobalt. When using this

assay Stowe (5) found that an ethanolic extract from the dwarf variety, Laxton's Progress, or Alaska peas greatly increased growth. The active compounds were believed to be a mixture of glycerides. In the course of the work it was found that certain "Tweens" (polyoxyethylene sorbitan esters of fatty acids) were almost as active as the ethanol extracts. The pea extract was only active when IAA was also present, and showed its maximum effect in the presence of IAA and GA_3.

Other interesting relations concerning lipophilic properties of plant growth substances have been noted in the past. Veldstra (6) pointed out that in most of the active growth substances there is a definite balance between the lipophilic properties of the ring and hydrophilic properties of the side chain, both of which are needed for activity. This observation suggested that the action involved physical bonding to a lipoidal material and an aqueous phase at the "active site".

Sell et al. have shown that the methyl or ethyl esters of IAA were approximately as active as IAA when 100 times more dilute (7). The hexyl thru nonyl esters were equally as active as IAA when 10 times as dilute. Esters containing more than 13 carbons in the alcohol portion were less active than IAA (8).

Nature of the Biochemical Problem

The course of this work involved the synthesis of steroidal esters of IAA to test the effect of a lypophilic moiety on the

activity of IAA esters. Sterols are found throughout the plant and animal kingdoms, but little is known about their metabolic role. Although these alcohols have somewhat the same nucleus, their individualities are clearly demonstrated by diverse differences in physical properties, absorption through membranes and physiological effect. Selectivity of absorption, for example, in the animal intestinal tract is good evidence that movement across membranes is not purely a physical phenomenon of diffusion, but well may have a sterochemical basis (9).

Esterification of IAA with 7 different sterols was attempted. The condensation of two, viz., stigmasterol and 7-hydroxycholesterol was not accomplished. Since only general methods of ester syntheses were available, much of the work involved finding proper reaction conditions.

NATURE OF THE SYNTHETIC WORK

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Synthesis of Esters

Perhaps the preparation of steroidal esters of IAA could have been effected by any one of the classical esterification methods. Weller et al. (8) reported the acid catalyzed condensation of IAA with alcohols up to 18 carbon atoms by dissolving the IAA in the anhydrous alcohol and adding dry hydrogen chloride.

The synthesis of 3-Indoleacetyl chloride (IACl) using phosphorous pentachloride and IAA at 0° was reported by Shaw and Wooly (10,11). First attempts to esterify IAA via IACl and the sterol, in dry pyridine, at 0° C. led only to failure. Norris and Rigby (12) described the preparation of t-butyl benzoate in 80% yield using this method. However, when an ethereal solution of IACl was added to pyridine containing the sterol, an orange tar-like material formed which would not go into solution, even when warmed. This material probably was a stable complex of pyridine and the acyl chloride. When IACl, in ether, was added to pyridine in the absence of a sterol, the same results were observed.

The method used was that of shaking a mixture of IACl, sterol, and silver carbonate at 30° C. in dry benzene. A condensation with cholesterol was attempted at 22° C. but only free sterol could be recovered. Repeating the experiment at 30° C. surprisingly accomplished the esterification. As a result of this preliminary work the syntheses in some cases were affected in refluxing petroleum

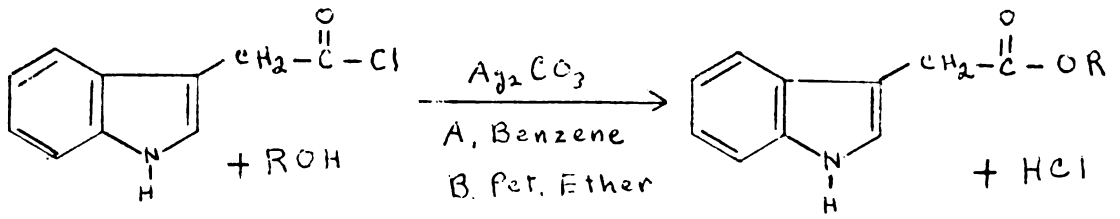
ether (40° - 45° C.). Hydrogen chloride was expelled from the reaction mixture or reacted with the silver carbonate, thus preventing decomposition of the IAA. Concentration after the reaction was an easy matter at a relatively low temperature.

IAA esters of cholesterol I, ergosterol II, and 7-dehydrocholesterol III were made by shaking in benzene, the latter two being condensed this way because of their unstability. Cholestanyl IV and β-sitosteryl V esters were made by refluxing in petroleum ether. The formulas are shown in figure 1.

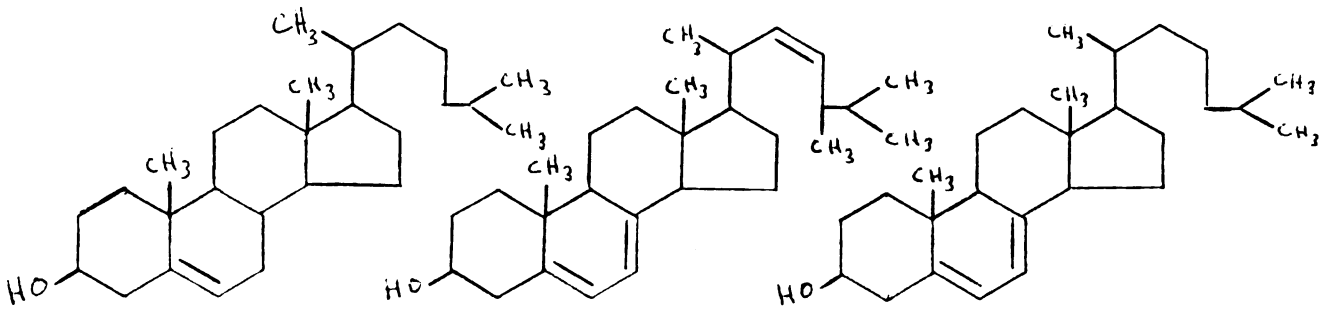
Many attempts were made to synthesize the IAA ester of stigmasterol VII. In the purification of the products a gelatinous material always formed, which could not be crystallized. Flakes, obtained once from chloroform-ethanol were subjected to a carbon and hydrogen analysis which resulted in values between those calculated for the ester and free sterol. After purification the gelatinous material formed once more.

Figure 1

Synthesis of IAA Esters



Sterols (ROH) Used in Method A.



I II III

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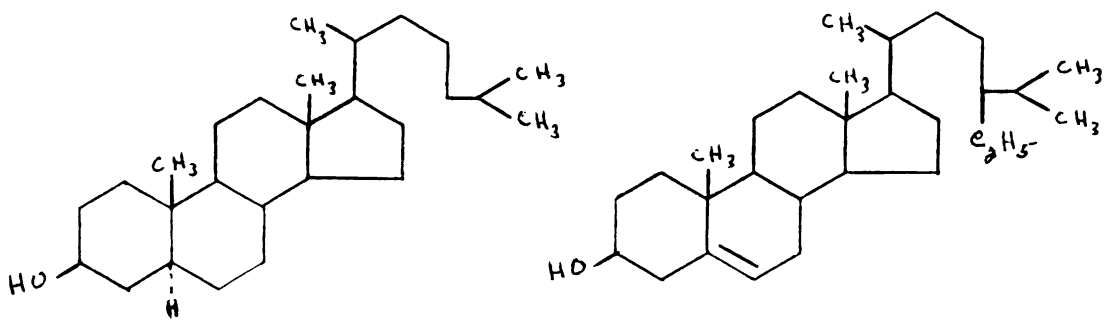
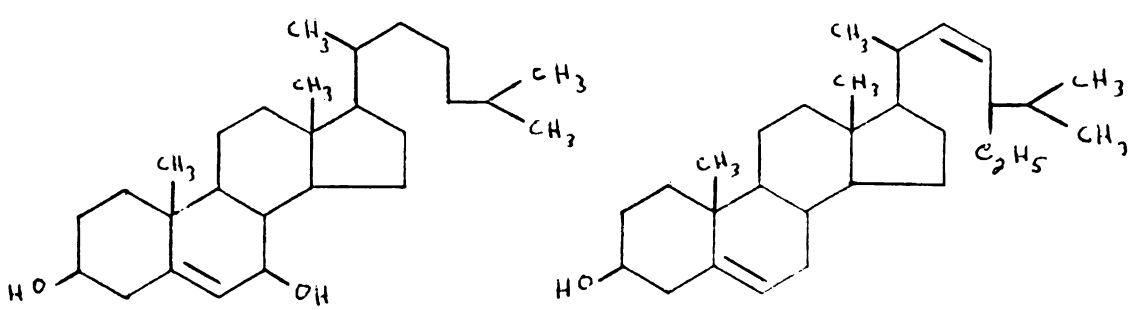


IV V

Sterols Not Esterified



VI VII

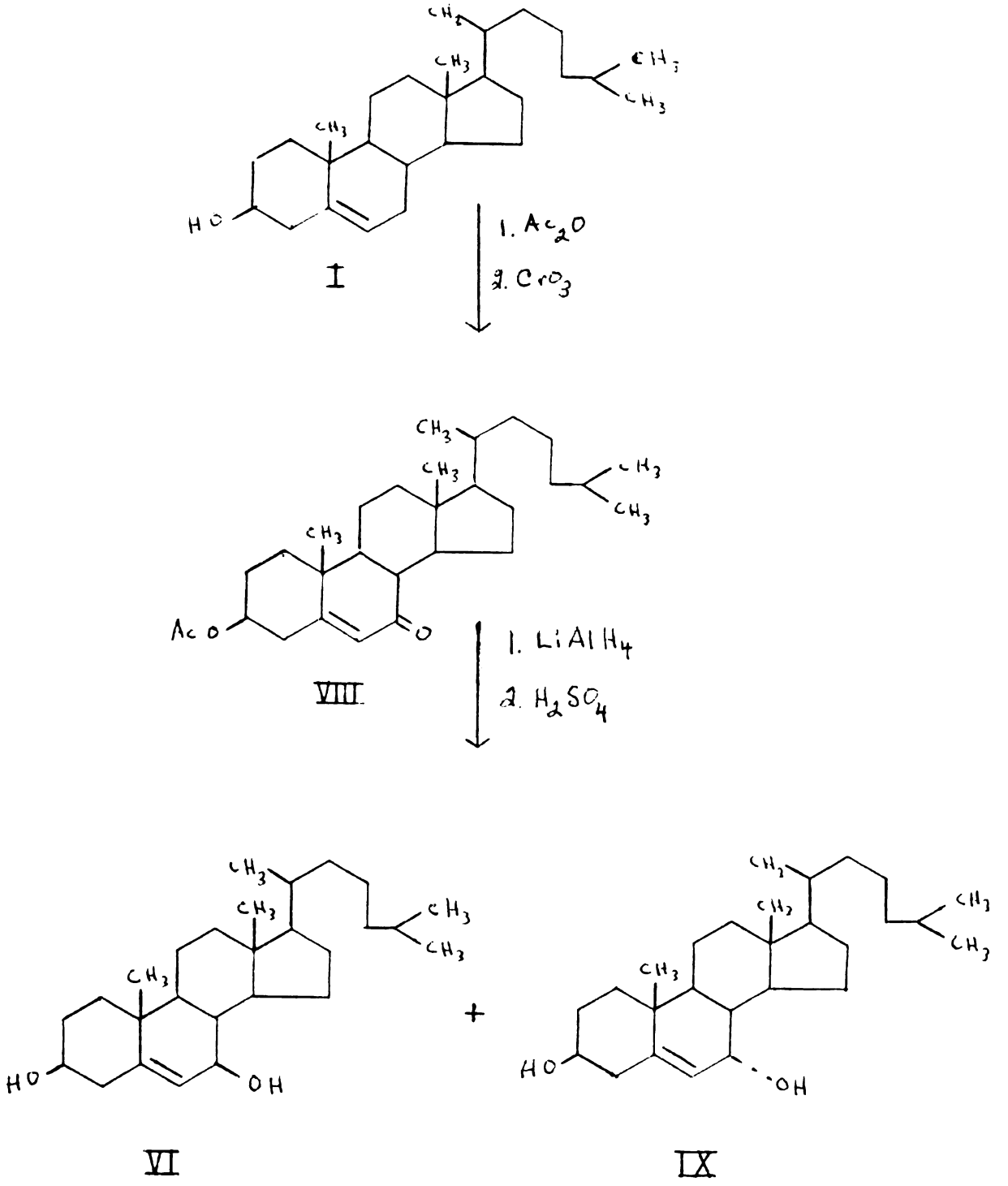
Synthesis of 7β-Hydroxycholesterol

7β-Hydroxycholesterol VI was made for the synthesis of the di-IAA ester. An outline of the method of preparation is shown in figure 2. 7-Ketocholesteryl acetate VIII was prepared as described by Fieser and Fieser (13) by oxidation of cholesteryl acetate (12) with chromic acid. The reduction of 7-ketocholesteryl acetate has been a point of confusion in the literature, in that two isomeric forms of the alcohol formed are possible. Windaus (15), using aluminum isopropoxide for the reduction, obtained a "7" hydroxycholesterol melting at 178° but failed to report the optical rotation. This product was presumed to be the α isomer until Wintersteiner (16) purified it by making the benzoates, recrystallizing and saponifying. He assigned to the epimer with the more positive rotation the (α) configuration rather than the usual trivial index, "α". The evidence is now quite strong that the epimer with the more positive rotation is of the (β) configuration (13).

The method (13) used for the reduction of 7-ketocholesteryl acetate utilized lithium aluminum hydride; the products being 95% of the (β) form and 5% of the (α) form. Two grams of impure 7β-hydroxycholesterol were obtained using lithium aluminum hydride, which when condensed with IACl only formed an oil that would not crystallize.

Figure 2

Synthesis of Epimeric 7-Hydroxycholesterols (13,14)



Purification of Esters

Purification of the products was one of the problems in the synthetic aspect of the work. It is well known that sterols form mixed crystals with ease, making complete purification by recrystallization very difficult. Advantage was taken of the fact that all of the sterols could be precipitated with digitonin leaving the esters in solution. Digitonin and the digitonide formed are insoluble in dry chloroform or ether, whereas the esters are soluble in these solvents (17). Thus, the esters were recrystallized as much as was practical, and then the digitonin treatment used to remove the last traces of free sterol.

EXPERIMENTAL

EXPERIMENTAL

Recrystallization of IAA

Ten grams of commercial IAA (167° - 170° C.[1]) was dissolved in 350 ml. of peroxide-free ethyl ether. The acid did not all dissolve and therefore the ether was filtered. To this solution, 200 ml. of dry petroleum ether[2] (40° - 45° C.) was slowly added resulting in the precipitation of a portion of the IAA. The solution was cooled over night and filtered giving 8.1g. of white crystals (169 - 170° C.). More of the acid was recovered by an additional precipitation with petroleum ether, but the product retained the yellow color of the crude material.

3-Indoleacetyl Chloride (10,11)

3-Indoleacetic acid (4.38 g., 0.025 mole, recrystallized) was dissolved in 150 ml. of anhydrous peroxide-free ethyl ether with stirring in a 3-neck round bottom flask. When solution was complete the flask was cooled to -12° C. or below with a salt-ice mixture. Phosphorus pentachloride (5.73 g., 0.027 mole) was now added in small portions over a period of 20 minutes, while maintaining as anhydrous conditions as possible. The reaction mixture was

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1. All melting points are uncorrected unless stated otherwise.

2. Washed with H_2SO_4, H_2O, dried over $CaCl_2$, and distilled over sodium.

stirred for an additional 15 minutes while the temperature raised to -10° C. The ether was quickly decanted from a small amount of unreacted PCl_5 into a 500 ml. round bottom flask and evaporated, with the aid of a vacuum pump and dry ice trap, to approximately 40 ml. Dry prechilled petroleum ether (400 ml., 40° - 45° C.) was added. After standing about one minute the solution was quickly filtered to remove a red amorphous material. The solution was cooled at -15° C. over night and concentrated under reduced pressure to 300 ml. bringing down 2.30 g. of whitish-pink crystals melting at 66° - 67° C. Concentrating the filtrate to 150 ml. gave 0.65 g. of a pink amorphous powder, total yield: 2.95 g. (64%). The preparation was varied by starting with 2, 4, 5, or 6 grams of IAA with the yields ranging from 50 - 68%. Drying of the IACl was carried out in vacuo over P_2O_5 and KOH. When storage for a short period was necessary (the acid chloride decomposes on standing), the compound was placed under vacuum in a desiccator in the cold.

Cholesteryl 3-Indoleacetate

Cholesterol was recrystallized twice from 95% ethanol and dried in vacuo at 100° C. Three grams (0.011 mole) of silver carbonate, 3.0 g. (0.0078) mole of cholesterol (m.p. 149° C.) and 3.0 g. (0.016 mole) of freshly prepared and dried IACl were shaken with 100 ml. of dry thiophene-free benzene for 18 hours. The acyl chloride and sterol dissolved leaving the silver carbonate as a suspension. During the reaction period the temperature was approximately 30° C.

The silver chloride and carbonate were removed by filtration and washed with dry benzene. After concentrating the filtrate to dryness under reduced pressure, the residue was treated with hot methanol, but a large portion failed to dissolve. The white to gray material was collected on a filter and air dried: 3.0 g. melting at 202° C. Upon cooling the methanol solution to room temperature 0.35 g. (m.p. 195° - 200° C.) of amorphous powder came out of solution. The total yield of crude product was 80% based on cholesterol.

One gram of material melting at 202° C. was treated with 0.8 g. of digitonin in 95% ethanol in the described manner. Recrystallization from warm chloroform - 95% ethanol gave 0.67 g. of needle-like crystals melting at 194.5° -195° C. (corrected); $[\alpha]_D^{23°}$ - 36° (c = .87 in $CHCl_3$); λ_{max}^{ETOH} 289, 280, 273 mμ, log ε 3.78, 3.86, 3.83. Anal. calcd. for $C_{37}H_{53}NO_2$: C, 81.72; H, 9.82; N, 2.58. Found: C, 82.02; H, 9.80; N, 2.66.

Ergosteryl 3-Indoleacetate

Two grams (0.0050 mole) of crystalline ergosterol, 2.0 g. (0.010 mole) of IACl and 2.0 g. (0.0072 mole) of silver carbonate were shaken with 100 ml. of dry thiophene free benzene for 39 hours. Silver residues were filtered off, washed with benzene and the filtrate concentrated in vacuo without warming. Nitrogen was passed through the solution to aid evaporation. Cooling from evaporation of the solvent caused an amorphous material to precipitate. The solid (0.84 g. fraction I) was filtered off and concentration of the

filtrate continued to give fraction II (1.0 g.). Additional removal of the solvent led to a brown tar that could not be purified.

Fraction I was recrystallized by solution in 10 ml. of benzene and addition of an equal volume of petroleum ether. Cooling several days brought out 0.4 g. of material melting at 174° - 176° C. Using the mother liquor and an additional 10 ml. of benzene the same procedure was attempted with fraction II, but no crystals would form. Evaporation to dryness, solution in 10 ml. of chloroform and addition of an equal volume of 95% ethanol gave 0.25 g. of white crystals melting at 170° - 172° C. Total yield of crude material, 0.65 g. or 23% based on ergosterol.

The two fractions (m.p. 174° - 176° C. and 170° - 172° C.) were combined and recrystallized from chloroform - 95% ethanol resulting in 0.55 g. of white mixed crystals.

The 0.55 g. of impure ester was treaded with 0.4 g. of digitonin in the usual manner. Recrystallization from chloroform at room temperature by allowing the solvent to slowly evaporate gave 0.32 g. of ester melting at 179° - 180° C. Microanalysis on this material resulted in the following values: found: C, 83.71; H, 9.94. Calcd. for ester: C, 82.41; H, 9.28. Calcd. for free sterol: C, 84.78; H, 11.18. These results indicated that free sterol was present, necessitating retreatment with digitonin. Using 0.5 g. of digitonin and the entire available ester, 0.2 g. of pearly leaflets melting at 179° - 180° C. (corrected) were obtained by final solution in dry ethyl ether, evaporation at room temperature

to 10 ml., and addition of the same volume of petroleum ether. Other physical constants: $[\alpha]_D^{23^\circ}$ - 76° (c = .80 in $CHCl_3$); λ_{max}^{EtOH} 290., 282., 272. mμ, log ε 4.10, 4.28, 4.26. Anal. Calcd. for $C_{38}H_{51}NO_2$: C, 82.41; H, 9.28; N, 2.53. Found: C, 82.25; H, 9.18; N, 2.66.

7-Dehydrocholesteryl 3-Indoleacetate

Purification of 7-dehydrocholesterol: The sterol was recrystallized from an ether-methanol mixture as suggested by Koch and Koch (18). By dissolving the yellow commercial solid in enough ether to accomplish solution and adding an equal volume of methanol, white crystals were obtained melting at 143° - 144° C.

Synthesis of ester: A mixture of 2.0 g. (0.0052 mole) of recrystallized 7-dehydrocholesterol, 2.0 g. (0.010 mole) of IACl and 2.0 g. (0.0072 mole) of silver carbonate was shaken in 100 ml. of dry, thiophene-free benzene for 38 hours. The mixture of silver chloride and carbonate was filtered off and washed with 20 ml. of benzene. Concentration in an atmosphere of nitrogen under reduced pressure forced a brown oil out of the solution. After removal of most of the benzene, the oil was transferred to a beaker and dissolved in enough chloroform to form a homogeneous solution. A stream of nitrogen now was passed over the solution, volatilizing the chloroform and cooling the mixture causing droplets of a brown oil to settle to the bottom of the beaker. The cold chloroform was decanted off the oil, effecting a good separation. No product could be obtained from the oil.

Ethanol (95%) was added to the chloroform decantate to precipitate white crystals which after two-fold recrystallization from chloroform - 95% ethanol resulted in 0.30 g. (11% based on sterol) of plates m.p. 169° - 170° C. (corrected); $[\alpha]_D^{23°}$ - 55° (c = .91 in $CHCl_3$); λ_{max}^{EtOH} 289.5, 281.5, 251. mμ, log ε 3.90, 4.03, 4.20. Anal. Calcd. for $C_{37}H_{51}NO_2$: C, 82.03; H, 9.49; N, 2.59; Found: C, 82.01; H, 9.27; N, 2.47.

Cholestanyl 3-Indoleacetate

Equal weights (3.94g.) of cholestanol (0.010 mole), IACl (0.020 mole) and silver carbonate (0.014 mole) were warmed in 110 ml. of dry petroleum ether (40° - 45° C.) in a three-neck 300 ml. round bottom flask fitted with a condensor and motor stirrer. A water bath maintained the stirred suspension at 40° - 45° C. during which time hydrogen chloride evolved and passed through a calcium chloride tube fitted to the condensor. After two hours of heating, 10 ml. of dry benzene was added to dissolve a precipitate forming in the reaction mixture. Hydrogen chloride production stopped after 3 hours of heating. Thirty ml. more of dry benzene was added and the water bath temperature raised to 55° - 60° for an additional 30 minutes. The solution was filtered in the hot to remove the silver residues. Upon cooling to room temperature, brown amorphous material formed which was filtered off and air dried (fraction I). Addition of petroleum ether to the filtrate brought down two more fractions (II and III).

Fraction I: 1.73 g., m.p. 155° - 160° C., dissolved in 80 ml. of absolute ethanol, treated with Norit A filtered and cooled to give .68 g., m.p. 177°.

Fraction II: 1.69 g., m.p. 173° - 177° C., dissolved in 50 ml. of absolute ethanol and cooled yielding 1.31 g., m.p. 175° - 177° C.

Fraction III: 1.48 g., m.p. 155° - 165° C., dissolved in 40 ml. of absolute ethanol and cooled giving 1.03 g., m.p. 174° - 177° C.

The filtrates from I, II and III were combined, concentrated under reduced pressure and cooled resulting in .50 g. of material, m.p. 175° - 176° C. This portion was combined with the precipitates from alcohol (total crude product: 3.52 g. or 64% based on cholestanol) and the entire product recrystallized once more from absolute ethanol giving 2.90 g., m.p. 174° - 176° C. One gram of this material was treated with 0.8 g. of digitonin. The final recrystallization was from chloroform - 95% ethanol. Glass-like plates were obtained: .52 g., m.p. 172° - 173° (corrected); $[\alpha]_D^{23^\circ}$ + 13 (c = .96 in $CHCl_3$); λ_{max}^{EtOH} 289.5, 279.5, 273., log ε 3.74, 3.82, 3.79. Anal. Calcd. for $C_{37}H_{55}NO_2$: C, 81.42; H, 10.16; N, 2.57. Found: C, 81.44; H, 10.16; N, 2.49.

β-Sitosteryl 3-Indoleacetate

The esterification was effected by refluxing a stirred solution of 3.19 g. (0.0077 mole) of β-sitosterol, 3.19 g. (0.017 mole) of IACl and 3.19 g. (0.012 mole) of silver carbonate in 100

ml. of dry petroleum ether (40° - 45° C.) in a 3-neck 300 ml. round bottom flask. A water bath (40° - 45° C.) was used to keep the solvent refluxing gently. After 3 hours of heating no more hydrogen chloride was produced so 40 ml. of dry benzene was added and the bath temperature raised to 45° - 50° C. until no more hydrogen chloride could be detected (about 2 more hours). The hot mixture was filtered and the silver precipitate washed with 25 ml. of ether.

The filtrate was concentrated under reduced pressure. Rapid evaporation and cooling precipitated a brown flocculent solid which then redissolved as the petroleum ether volatilized. The volume was reduced to 80 ml., 200 ml. of petroleum ether was added, and the precipitating material cooled in the refrigerator over night. Filtering and drying the product gave a yellow-brown mass weighing 3.40 g. and melting at 160° - 165° C. (fraction I).

After evaporating the filtrate to dryness, the residue was dissolved in 95% ethanol, treated with Norit A and cooled to give a gray amorphous powder melting at 155° - 165° C. (fraction II).

Fraction I was recrystallized from ether-petroleum ether several times resulting in a number of fractions, each of which melted over a range of about 5 degrees. Recrystallization from this solvent system raised the melting point and removed the brown color, but micro-analysis of a sample melting at 177° - 178° C. gave the following results: Found: C, 83.02; H, 10.01 (average of three determinations); Calcd. for ester: C, 81.91; H, 10.05. Calcd. for free sterol: C, 83.99; H, 12.15. Free sterol was obviously present.

The sterol free ester was obtained by treating .2 g. of material melting at 176° - 177° C. with 0.4 g. of digitonin. A white powder was precipitated out of an ethyl ether solution with petroleum ether. After two-fold recrystallization from 95% ethanol enough material was obtained for microanalysis melting at 152° - 153° C. Calcd. for $C_{39}H_{57}NO_2$: C, 81.91; H, 10.05. Found: C, 81.69; H, 9.93.

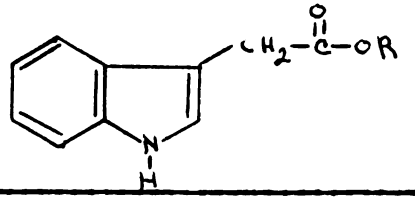
All of the previous fractions (including fraction II) were recrystallized from 95% ethanol resulting in 1.30 g. of crude material which was treated with 2. g. of digitonin. Two recrystallizations from 95% ethanol gave 0.3 g. of white fluffy flakes melting at 153° - 154° C. (corrected) representing 7.0% yield. Carbon and hydrogen analyses were not repeated because of the similarity in melting point with the previous material. Anal. for N: Calcd., 2.45, Found, 2.54; $[\alpha]_D^{23°}$ -32° (c = .88 in $CHCl_3$); λ_{max}^{EtOH} 289.5, 280., 274., log ϵ 3.76, 3.84, 3.82.

Purification of Esters (15)

The impure ester was dissolved in ether or chloroform (approximately 15 ml./g. of ester). This solution was added to digitonin dissolved in 95% ethanol. Calculation of the digitonin (M.W. 1229.) needed was based on the fact that the digitonide contains a molar ratio of sterol:digitonin of 1:1 (19). The ethanolic solution was filtered to remove precipitated digitonide and digitonin. The filtrate was concentrated and dissolved in anhydrous peroxide-free ether.

TABLE I

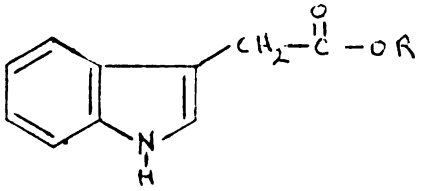
SUMMARY OF MICROANALYSES OF ESTERS OF IAA



R	Emperical Formula	Calcd. for ROH %		Calcd. for Ester %			Found for Ester		
		C	H	C	H	N	C	H	N
Cholesteryl	$C_{37}H_{53}NO_2$	83.87	11.99	81.72	9.82	2.58	82.02	9.80	2.66
Ergosteryl	$C_{38}H_{51}NO_2$	84.78	11.18	82.41	9.28	2.53	82.25	9.18	2.66
7-Dehydro-cholesteryl	$C_{37}H_{51}NO_2$	84.31	11.53	82.03	9.49	2.59	82.01	9.27	2.47
Cholestanyl	$C_{37}H_{55}NO_2$	83.43	12.45	81.42	10.16	2.57	81.44	10.16	2.49
β -Sitosteryl	$C_{39}H_{57}NO_2$	83.99	12.15	81.91	10.05	2.45	81.69	9.93	2.51

TABLE II

SUMMARY OF PHYSICAL CONSTANTS OF ESTERS OF IAA



R	M.P.C. (Corrected)	$[\alpha]_D^{CHCl_3}$	Absorption λ_{max}^{EtOH} (mμ)	log ε
Cholesteryl	194.5° - 195°	-36°	289.	3.78
			280.	3.86
		c = .87	273.	3.83
Ergosteryl	179° - 180°	-76°	290.	4.10
			282.	4.28
		c = .80	272.	4.26
7-Dehydro-cholesteryl	169° - 170°	-55°	289.5	3.90
			281.5	4.03
		c = .91	251.	4.20
Cholestanyl	172° - 173°	+13°	289.5	3.74
			279.5	3.82
		c = .96	273.	3.79
β-Sitosteryl	153° - 154°	-32°	289.5	3.76
			280.	3.84
		c = .88	274.	3.82
H			280.	3.80

Check on methodology; from lit. 280 mμ log 3.80 (20).

Digitonin, which does not dissolve, was filtered off and the ether solution concentrated to dryness, leaving the pure ester.

Bioassay (7)

The insolubility of the steroidal esters of IAA in aqueous solvents limited the methods of bioassay. Lipoidal substances can be assayed by dispersing the compounds in lanolin and applying the dispersion to an unfertilized ovary. The increase in the diameter of the ovary is used as a measure of activity.

The IAA esters, β -sitosterol, ergosterol and IAA (standard) were weighed in 10 ml. volumetric flasks and made to volume with anhydrous peroxide-free ether. All of the compounds went into the ether well, except ergosterol 3-indoleacetate which was transferred to a 100 ml. flask. An aliquot of the ether solution was pipetted into a test tube containing 1 g. of warm anhydrous lanolin previously measured from a calibrated dropper. The pipetted ether was mixed well with the lanolin and warmed until evaporated, leaving the compound as a dispersion. Dilutions of the original solution were made so that the final concentrations for each of the esters were .1 - .00001 molar in the lanolin. The IAA and free sterol concentrations were .1 - .00001 molar.

The bioassay was carried out in the following manner: Stamens were removed from unopened flowers of tomato plants (variety, Michigan-Ohio Hybrid F_1), thus exposing the ovary. Two replications with two or three flowers on each of the plants were used. An excess of

lanolin dispersion was applied to the exposed ovary, and after 7 days their diameters measured.

RESULTS AND DISCUSSION OF THE BIOASSAY

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None of the compounds tested induced parthenocarpic fruit development as well as IAA. Table IIIshows the results obtained. Cholesteryl, cholestanyl and β-sitosteryl esters as well as the free ergosterol and β-sitosterol showed no activity. 7-Dehydrocholesteryl and ergosteryl esters responded at .1 and .01 molar respectively.

Interpretation of the activity of compounds in a particular bioassay is not a simple matter. Suggestions have been made concerning the activity of esters of IAA. Using the avena curvature test as a criterion of activity Kögl and Kostermans (21) showed that the methyl, ethyl, propyl or isopropyl esters of IAA were less active than the parent acid, and that activity decreased with increasing size of the alcohol portion. Assuming that the ester must be hydrolyzed *in vivo* to exert its effect, it has been suggested that the difficulty of hydrolysis increases with the size of the alkyl moiety (21,22). Perhaps impeded transport is also important (23).

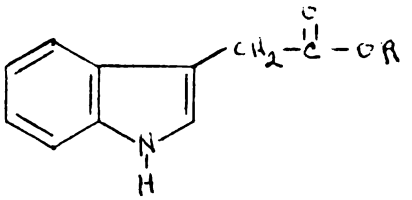
The secondary factors mentioned above could well be operating with the steroidal esters of IAA. In addition,the obvious possibility exists that a large increase in size and molecular weight would hinder the movement across the cell wall where hydrolysis, if necessary, would have to take place. However, there is no direct evidence for this interpretation.

The slight activity of 7-dehydrocholesteryl and ergosteryl

esters indicates that they can pass across the cell membrane. Free IAA in these two esters in small enough amounts to elicit a biological response but not enough to alter the microanalysis might cause similar results. In that the esters were recrystallized from chloroform-ethanol this possibility seems unlikely because of the solubility of IAA in each of these solvents. It is interesting to note that one unique feature of both esters is that in the sterol portions there is a 7-8 double bond.

TABLE III

ACTIVITY OF ESTERS OF IAA IN THE INDUCTION OF PARTHENOCARPIC FRUIT DEVELOPMENT



	Parthenocarpic Activity; Molarity				
R	.1	.01	.001	.0001	.00001
H (Standard)	++	++	+	-	0
Cholesteryl	-	-	-	-	-
Ergosteryl	0	+	-	-	-
7-Dehydrocholesteryl	+	-	-	-	-
Cholestanyl	-	-	-	-	-
β-Sitosteryl	-	-	-	-	-
Free Sterols					
Ergosterol	-	-	-	-	0
β-Sitosteryl	-	-	-	-	0

The number of plus signs represents the relative activity

0 Not tested

- No activity

SUMMARY

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3-Indoleacetic acid esters of cholesterol, ergosterol and 7-dehydrocholesterol were synthesized from the acyl chloride and sterol in dry benzene. Esterification of 3-indoleacetic acid with cholestanol and β-sitosterol was accomplished via the acyl chloride and sterol in anhydrous petroleum ether.

The cholesterol, cholestanol and β-sitosterol esters displayed no activity in the parthenocarpic fruit development bioassay. 3-Indoleacetic acid esters of ergosterol and 7-dehydrocholesterol were less active than the free acid.

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