Reaction of Ergosteryl Acetate with Maleic Anhydride and Preparation of 5,7-Ergostadien-3β-ol[1,2]

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ABSTRACT

An improved, large scale synthesis of the ergosteryl acetate-maleic anhydride Diels-Alder adduct and its pyrolysis are described. The complex mixture obtained by reaction of the two constituents was refluxed with methanol to convert the succinic anhydride derivatives, formed by the "ene" reaction, to soluble half esters, leaving the insoluble Diels-Alder adduct largely unchanged. The latter was hydrogenated and pyrolyzed in vacuo to yield 5,7-ergostadienyl acetate together with lesser quantities of the acetates of 7,9(11)-ergostadien-3β-ol, 6,8(9)-ergostadien-3β-ol, and 8,14-ergostadien-3β-ol. These components were separated and purified by argentation column chromatography and crystallization.

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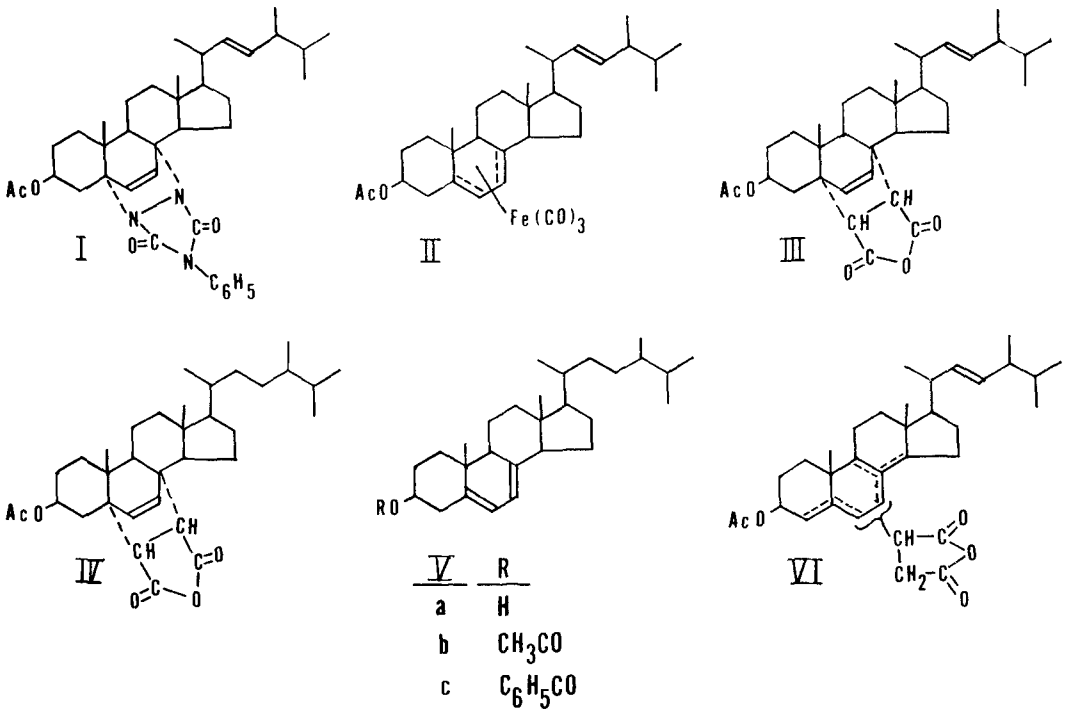
INTRODUCTION

We required 5,7-ergostadien-3β-ol (Va; Scheme 1) for our studies on sterol utilization by species of *Drosophila*. A diene synthesis (1) applied to 22,23-dihydrobrassicasteryl acetate was considered and rejected because preparation of this sterol in a large quantity is time consuming (2,3). The hydrogenation of diene blocked derivatives of ergosterol seemed to be a better route.

At first, the triazoline dione adduct (I; Scheme 1) appeared ideal for this purpose. The derivative is obtained in good yield and can be converted readily back to ergosterol with $LiAlH_4$ (4). Hydrogenation of I (Scheme 1) over palladium, platinum or Raney nickel, however, always reduced the Δ^6 bond before the Δ^{22} was attacked (A. Wilkinson, unpublished



Scheme I. Formulas of adducts and products.

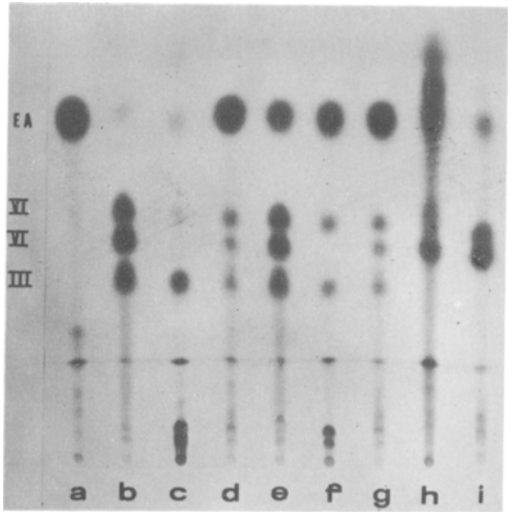


FIG. 1. TLC, System A. a = Ergosteryl acetate (EA); b = EA-maleic anhydride (MA) reaction in inert solvents; c = EA-MA reaction in polar solvents; d = EA-MA reaction, 4:1 ratio of reactants, respectively; e = EA-MA reaction in 25 ml benzene; f = EA-MA reaction with 0.025 ml triethylamine; g = EA-MA reaction, room temperature, 4 days; h = same as g plus BF_3; i = same as g plus $AlCl_3$. III,VI = see Scheme 1.

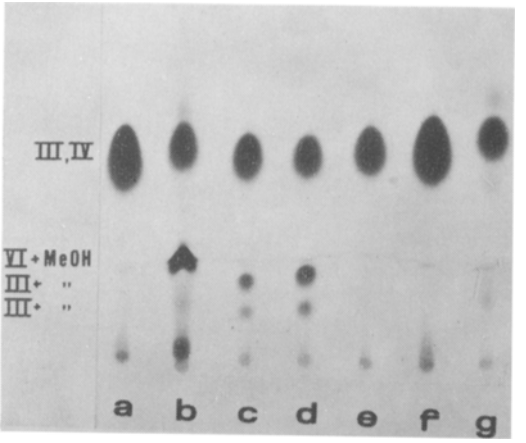


FIG. 2. TLC System A. a = Adduct III, 3 days, 170 C, with and without maleic anhydridie (MA); b = ergosteryl acetate (EA)-MA reaction products refluxed 4 hr in methanol; c = adduct III refluxed overnight in methanol; d = crude EA-MA adduct III; e = purified adduct III; f = 22,23-dihydroadduct IV; g = 6,7; 22,23-tetrahydroadduct. III,IV,V = see Scheme 1.

data). After our work was completed, similar results were reported (5).

The iron tricarbonyl complex (II; Scheme 1) was prepared next (6). It tenaciously resisted hydrogenation over palladium, even at 150 C and 14 atm. Use of Raney nickel destroyed the yellow complex at room temperature and gave 7,22- and 7-derivatives, the typical products of hydrogenation of ergosteryl acetate (EA) over nickel (A. Wilkinson, unpublished data).

We then turned to the classical synthesis of 5,7-ergostadienylacetate (Vb; Scheme 1) via the maleic anhydride (MA) adduct (III; Scheme 1) and its dihydro derivative (IV; Scheme 1) (7). This procedure has been used frequently for the preparation of 22,23-dihydroergosteryl acetate and the free sterol (8-12), but experimental details and physical constants are missing in several of these reports. A number of related studies (13-19) discussed the EA reaction with MA and described products of the general formula (VI; Scheme 1) formed by the "ene" reaction (20) between the reactants.

In this paper we report further studies on the reaction of EA with MA, a simple procedure for the large scale preparation and purification of the Diels-Alder adduct (III; Scheme 1), its hydrogenation and pyrolysis, and the isolation of Vb (Scheme 1), and three other ergostadienyl acetates from the pyrolytic reaction mixture.

EXPERIMENTAL PROCEDURES

EA was recrystallized from chloroform-methanol, MA was sublimed at atmospheric pressure, and thiophene free benzene was dried over sodium and distilled. Sealed tube reactions were run under N_2 in an oil bath. Autoclave reactions were run in the 1 liter Parr Series 4500 pressure apparatus. Melting points are corrected and were taken in vacuo in capillary tubes with a Thomas-Hoover apparatus. Systems for TLC were: A) chloroform:acetone: acetic acid (97:2:1), silica gel plates: B) chloroform:acetone (98:2), 10% $AgNO_3$-silica gel plates; 30% H_2SO_4 spray. GLC with 5% OV-101 on Anachrom ABS, 260 C; relative retention times of ergostanyl acetate derivatives (cholesteryl acetate = 1.00) were: $\Delta^{8(14)}$ = 1.26; Δ^5 = 1.27; $\Delta^{7,9(11)}$ = 1.35; $\Delta^{8,14}$ =1.36; $\Delta^{6,8(9)}$ = 1.36; $\Delta^{5,7}$ = 1.36; $\Delta^{5,7,22}$ = 1.20. UV spectra in 95% ethanol were obtained with the Perkin-Elmer 202.

Preparation of EA-MA Adduct III

A. Preliminary experiments:

1. EA and MA (200 mg, each) in 5 ml inert solvent (ethyl acetate, 1,2-dichloroethane, carbon disulfide, mesitylene [Matheson Coleman and Bell, Los Angeles, CA], Skellysolve B [Skelly Oil Company, Tulsa, OK], tetrahydrofuran, carbon tetrachloride, chloroform, acetic acid, benzene, xylene [Mallinckrodt Chemical Works, St. Louis, MO], acetonitrile, mono-, di- and triglyme, acetic anhydride [Aldrich Chemical Company, San Leandro, CA]) or no sol-

vent were heated in sealed tubes 3 hr at 180 C. The tubes were cooled, opened, and the contents assayed by TLC (Fig. 1b).

2. The same reaction (Experiment 1) was run in 5 ml polar solvents (dimethyl formamide, dimethyl acetamide, ethylene carbonate, dioxane, pyridine, collidine [Aldrich Chemical Co., San Leandro, CA]). The reaction mixtures were poured into water and the products extracted with benzene and assayed by TLC (Fig. 1c).

3. EA (200 mg) and MA (¼ to 3 moles/mole EA) were heated at 180 C, for 3 hr in benzene (Fig. 1d).

4. EA (200 mg) and MA (100 mg) were heated at 180 C for 3 hr in 5-25 ml benzene (Fig. 1e).

5. EA (200 mg) and MA (100 mg) in 5 ml benzene were heated at 180 C for 3 hr with 0-0.1 ml triethylamine (Fig. 1f).

6. EA and MA (200 mg, each) in 5 ml benzene, N_2, 25 C, 4 days (Fig. 1g).

7. Same as Experiment 6, with 0.5 ml BF_3 etherate (Fig. 1h).

8. EA and MA (200 mg, each) in 5 ml ethylene chloride, 6.6 mg sublimed $AlCl_3$ added, room temperature, 4 days (mole ratios 1 EA:4 MA:0.1 $AlCl_3$) (Fig. 1i).

9. Adduct III (50 mg) in 3 ml benzene, sealed tube, N_2 170 C, 3 days with and without 50 mg MA (Fig. 2a).

10. Products from a typical EA-MA reaction in benzene (Fig. 1b) were refluxed with methanol for 4 hr (Fig. 2b).

11. Adduct III (700 mg) in 50 ml methanol was refluxed overnight (Fig. 2c).

B. Large scale preparations: EA (100 g) and MA (50 g) in 600 ml benzene were heated under N_2 in the autoclave for 20 hr at 150 C. The yellow charge transfer complex that formed immediately between the reagents gradually disappeared as the reaction progressed. After cooling and addition of Celite (Johns-Manville, Denver, CO), the benzene solution was filtered and solvent removed in vacuo in a 2 liter flask. Methanol (850 ml) was added to the residue (Fig. 1b), and the suspension refluxed 4 hr to convert the "ene" reaction products (VI; Scheme 1) to mono-methyl esters (Fig. 2b). The resulting mixture was cooled overnight at 4 C after which the prisms of the Diels-Alder adduct III (Scheme I) were removed and dried (Fig. 2d) (mp = 215-218 C). Yield from five such runs was 132 g (21.5%). The combined products were recyrstallized from 2 liters acetone to give 127 g pure III (Scheme 1) (Fig. 2e) with a mp = 219.5-220.5 C. In the literature, the mp is reported as: 210-212 C (9); 216 C (8,14); 216-218 C (16,18); 217.5-218 C (11); and 217-220 C (19). The compound pyrolyzed in the injection port of the gas chromatograph (290 C) to give a single peak corresponding in retention time to that of EA.

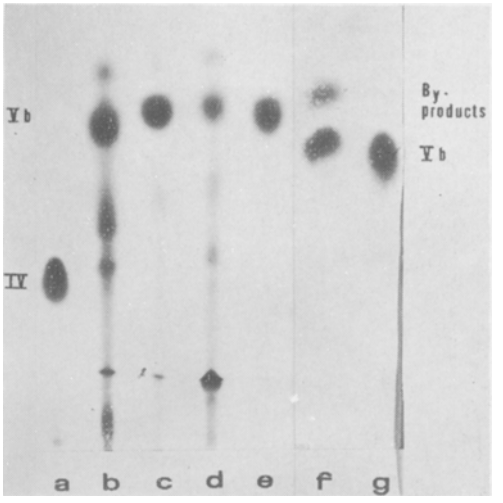


FIG. 3. TLC System A (left side). a = Dihydro-adduct IV; b = sublimate from pyrolysis of IV; c = methanol insoluble ergostadienyl acetates; d = methanol soluble products; e = crude 5,7-ergostadienyl acetate. TLC, System B (right side). f = Crude 5,7-ergostadienyl acetate; g = purified 5,7-ergostadienyl acetate. IV,V = see Scheme 1.

22,23-Dihydro adduct IV

Adduct III (50 g) in 1 liter distilled ethyl acetate was stirred overnight at room temperature and atmospheric pressure with 10% Pd on carbon (4 g) and hydrogen. Catalyst and solvent were removed to leave IV (Scheme 1) as an amorphous solid (Fig. 2f), (sinters = 172 C, melts = 203.5-205 C). The literature report sinters = 172-174 C, melts = 202-203 C (14). All attempts to crystallize IV from many solvents failed. The compound also pyrolyzed in the gas chromatograph and gave a single peak corresponding in retention time to that of 22,23-dihydroergosteryl acetate (Vb; Scheme 1).

6,7;22,23-Tetrahydro adduct

Adduct III (0.5 g) in 50 ml ethyl acetate containing five drops of 70% $HClO_4$ was stirred with PtO_2 (0.15 g) and hydrogen for 2 weeks. Catalyst was removed, the solution washed with aqueous sodium acetate and evaporated. The residue was crystallized from methanol to yield 0.2 g tetrahydro EA-MA Diels-Alder adduct (Fig. 2g) with a mp = 186-188 C. In the literature, the mp is reported to be 187-187.5 C

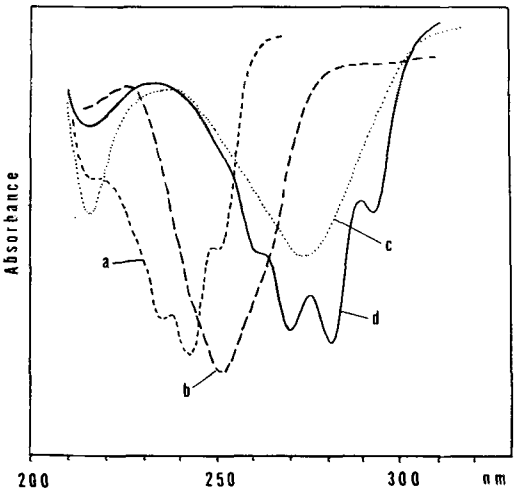


FIG. 4. UV spectra of: a = 7,9(11)-ergostadien-3β-ol; b = 8,14-ergostadien-3β-yl acetate; c = 6,8(9)-ergostadien-3β-ol; d = 5,7-ergostadien-3β-ol.

(14). The tetrahydro adduct gave no peak on GLC and was stable to pyrolysis (220 C, 6.5 hr, in vacuo).

Pyrolysis of dihydro adduct IV

A magnetic stirring bar and 10 g IV (Scheme 1) (Fig. 3a) were placed in each of 2 1-liter filter flasks which were closed with a rubber stopper and attached to a vacuum manifold. The system was evacuated several times and flushed with N_2, a water aspirator vacuum applied (32 mm), and the flasks immersed to a depth of 4 cm in 220 C oil baths on magnetic stirrer hot plates. After 5 hr, an oil pump vacuum was applied (0.2 mm) and the products of pyrolysis sublimed to the cooler walls of the flasks for 2 days. After cooling, N_2 was admitted to the system and the sublimates (Fig. 3b) removed from the unsublimated residues with benzene. Evaporation of the benzene left a solid residue (80 g) that was suspended in 800 ml methanol and refluxed 4 hr under N_2. The mixture was cooled at 4 C overnight and filtered (Fig. 3c,d). The precipitate was refluxed briefly under N_2 with 250 ml acetone, the mixture again cooled overnight, and filtered to give 40 g crude 5,7-ergostadienyl acetate (Vb; Scheme 1); mp =155-156 C; ϵ_{282} = 9,200, ca. 80% pure (Fig. 3e,f).

Purification of 5,7-ergostadienyl acetate (Vb)

Four kg 100 mesh SiO_2 (Mallinckrodt Chemical Co., St. Louis, Mo.), 800 g $AgNO_3$, and 2 kg Super-Cel (Johns-Manville, Denver, CO) were slurried with 3 liters water and dried on the steambath and at 110 C. The mixture was screened (80 mesh) and activated overnight at 110 C. Typically, 1 kg of this mixture was poured into 6 x 120 cm chromatographic tubes with 5% ether in low boiling petroleum ether (Skellysolve F, Skelly Oil Co., Tulsa, OK), the steryl acetate samples placed on the columns (10 g in 30 ml benzene), and the columns eluted with 5% ether in Skellysolve F. Fractions (500-700 ml) were collected, evaporated at atmospheric pressure under N_2. The residues were analyzed by GLC, TLC, and UV. After a run, the columns were eluted with ether, the packing dried, rescreened, reactivated, and reused.

A single passage of crude Vb through the tubes gave the fractions shown in Table Ia. Six more runs on pooled materials gave the fractions also shown in Table Ib. The two purest samples (D and E, Table I) were recrystallized from ethanol to give 3.45 g Vb (97.3% purity by UV) and 9.95 g Vb, respectively; mp = 162-163 C; ϵ_{282} = 11,400 (98.4% purity). A sample of the latter was recrystallized from acetone to yield Vb (Fig. 3g); mp =162.5-163.5 C; ϵ_{282} = 11,560 (99.7% pure). The literature report the mp as 155-157 C (11), 157-158 C (7), 160-162 C, 165-166 C (9). As a standard for UV, a chromatographically pure sample of 7-dehydrocholesteryl acetate (mp = 149-149.5 C) exhibited an ϵ_{282} = 11,600 with the spectrophotometer.

5,7-Ergostadien-3β-ol (Va)

A portion of Vb was hydrolyzed under N_2 with alcoholic KOH and the product recrystallized from acetone to give long needles of Va monohydrate, with a mp = 161.5-162.5 C, and ϵ_{282} = 11,600 (Fig. 4d). In the literature the mp= 150-151 C (9), 152-153 C (7,12), and 153-154 C (8).

5,7-Ergostadien-3β-yl benzoate (Vc)

Va (200 mg) in 5 ml pyridine were stirred under N_2 while 3 0.2-ml portions of benzoyl chloride were added over 30 min. Stirring was continued for 2 hr, after which time 50 ml methanol was added to decompose excess benzoyl chloride, solubilize the pyridinium hydrochloride, and precipitate the steryl benzoate (Vc; Scheme 1). The latter was recrystallized from acetone-benzene, with a mp = 162.5-163 C. The mp as cited in a previous study (9) is 156-157 C.

Separation of byproducts formed during pyrolysis of IV

Fraction B (13.4 g; Table Ib) was placed on a 1 kg silver nitrate column and eluted with 1% ether in Skellysolve F. Eluates (300 ml) from

TABLE I

Silver Nitrate Column Purification of Crude 5,7-Ergostadienyl Acetate (40 g)

	a		b	
Fraction	Wt (g)	Purity by UV	Wt (g)	Purity by UV
A	0.52	Byproducts	1.17	Oily impurity
B	3.55	Byproducts	13.75	Byproducts
C	11.17	60%	1.01	60-80%
D	13.25	80-90%	4.43	>93%
E	8.71	>95%	12.48	>95%
F	0.96	ca. 90%	5.48	Waxy ether eluate from columns
Recovery	38.20		38.30	

the column were analyzed by UV; all of the diene byproducts had the same retention time on GLC and the same R_f on TLC. The following crude fractions were obtained A) 1.51 g, rich in 7,9(11)-ergostadienyl acetate; B) 3.16 g, rich in 6,8(9)-ergostadienyl acetate; and C) 1.3 g, rich in 8,14-ergostadienyl acetate. The compounds were purified and identified as follows.

7,9(11)-Ergostadien-3β-ol. The impure acetate was hydrolyzed and free sterol purified on a 20% silver nitrate alumina column with 20% ether in Skellysolve F. The sterol was recrystallized from methanol, with a mp = 145.5-146.5 C, and ϵ_{236} = 14,300, ϵ_{243} = 16,200, and ϵ_{251} =10.800 (Fig. 4a). As cited in the literature, mp = 149-151 C (11), ϵ_{242} = 9,400 (11). Dorfman (21) lists ϵ_{236} = 14,200, ϵ_{243} = 15,700, and ϵ_{251} = 10,900 for 7,9(11),22-ergostatrien-3β-ol.

6,8(9)-Ergostadien-3β-ol. The impure acetate was recrystallized several times from acetone, hydrolyzed, and the free sterol recrystallized from acetone and ethanol, with a mp = 158-159 C, and ϵ_{273} =5,000 (Fig. 4c). The compound has never been reported. Its structure was deduced from the UV spectrum; ϵ_{275} = 4,700 for 6,8(9)-coprostadienol and ϵ_{275} = 5,300 for 6,8(9)-cholestadienol (21). The free sterol partially decomposed on a silver nitrate alumina column.

8,14-Ergostadien-3β-yl acetate. The impure acetate was rechromatographed on a 20% $AgNO_3$ silica gel column with 1% ether in Skellysolve F and recrystallized many times from methanol. The 8,14-acetate was enriched in the methanol soluble fractions. Eventually a chromatographically pure sample was obtained, (mp = 136-137.5 C, λmax 251 nm) (Fig. 4b). As cited in the literature, the mp = 136-138 C, λmax 251 nm (22). The 8,14-acetate was hydrogenated over Pd in ethyl acetate-acetic acid to 8(14)-ergosten-3β-yl acetate, with a mp = 109.5-111 C. As cited in the literature, mp = 110-111 C (23). The free diene also partially decomposed when purification was attempted on a silver nitrate alumina column.

DISCUSSION

The composition of the product mixture obtained by reaction of EA with MA appeared to be insensitive to the conditions of the reaction. The same ratio of products formed, III vs VI as assessed by TLC, when the reaction was run in all types of inert solvents (Fig. 1b), when 0.25-3 moles MA were used/mole EA (Fig. 1d), and when the concentration of the reagents was varied (Fig. 1e). Although the reaction was inhibited in polar solvents and amines (Fig. 1c), partial inhibition by very small amounts of triethylamine did not prevent formation of "ene" reaction products (Fig. 1f). Even at room temperature, where the reaction was very slow (13), the usual ratio of products still formed (Fig. 1g).

The Diels-Alder additions of MA to aromatic dienes and of α,β-unsaturated carbonyl compounds to butadienes are catalyzed by Lewis acids (24-26). Neither $AlCl_3$ nor BF_3 were of any value in our case, however; the rate of isomerization of EA by these reagents canceled any catalytic effect they might have had on the Diels-Alder reaction (Fig. 1h,i).

To determine whether the "ene" products (VI) were produced by rearrangement of III, the latter was heated in benzene for 3 days at 170 C with and without MA. No changes were noted (Fig. 2a), showing that once formed, III is stable to the reaction conditions. These experiences, plus those of others (14,16-18), indicate that a 20-25% yield of III is the best attainable from the reaction of EA with MA.

At first, we isolated III by crystallization

(14) or column chromatography (9), but these methods were tedius and did not readily give pure samples. By chance we noted that when the reaction mixture (Fig. 1b) was crystallized from hot methanol, a portion of the "ene" products was converted to materials having a lower R_f on TLC. When the mixture was refluxed 4 hr in methanol, virtually all of the "ene" products were converted to more polar compounds (Fig. 2b). The reaction of methanol with the mixture of monosubstituted succinic anhydrides VI to form methyl hydrogen succinates is apparently much faster than its reactions with the rigid, more hindered, disubstituted succinic anhydride III. Even when III was refluxed 18 hr with methanol, less than half of the material was converted to a mixture of two monoesters (Fig. 2c).

This led to a convenient preparation of III in large quantities without fractional crystallizations or column chromatography. EA can be heated with excess MA in a number of common solvents under N_2 until all of the sterol has reacted (Fig. 1b). The solvent is then removed, and the residue refluxed with methanol for 4 hr to react with and solubilize the "ene" products (VI). The Diels-Alder adduct (III) reacts only to a negligible extent under these conditions and remains insoluble (Fig. 2d). It is then crystallized from acetone to remove traces of impurities (Fig. 2e).

Hydrogenation of III to IV (Fig. 2f) over Pd goes smoothly; overhydrogenation is not a problem. Even if a little occurs, the tetrahydroderivative (Fig. 2g) is stable to pyrolysis. Underhydrogenation of III can be detected by GLC. Pyrolysis of III and IV in the injection port of the gas chromatograph yields EA and its 22,23-dihydroderivative, two sterols that are easily separated by this method.

Many small scale experiments on the pyrolysis of the dihydro adduct (IV) led to the method described in the previous section. Pyrolyses in solution (squalene or dibutyl phthalate), in benzene containing cyclopentadiene, at various temperatures (190-240 C) and times, and in different types of apparatus were all tried. None of the methods prevented formation of anhydride containing byproducts (Fig. 3b). These, however, could be removed readily from the mixture of ergostadienyl acetates by refluxing the pyrolyzate in methanol. The anhydrides were converted to methyl hydrogen esters and dissolved (Fig. 3d) in the alcohol, whereas, the steryl acetates remained largely insoluble (Fig. 3c). It could not be determined whether the anhydrides were derived from rearrangements of IV at 220 C or in a recombination of released MA with Vb or other dienes by the "ene" reaction.

Separation of 5,7-ergostadienyl acetate from the other diene acetates by multiple recrystallizations was uneconomical. Chromatography of the crude steryl acetate mixture (Fig. 3e,f) on silver nitrate columns led to Vb in 93-95% purity, after which it could be crystallized to 99.7% purity without excessive loss of material. Chromatography of 5,7-dienes on silver nitrate columns led to some losses (27), but these were minimized by exclusion of air, where possible, and by working with acetates (28).

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