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MECHANISM OF THE ENZYMATIC CLEAVAGE OF THE 9β,19-CYCLOPROPANE RING OF CYCLOEUCALENOL

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Key Word Index—*Zea mays*; Gramineae; biosynthesis of plant sterols; cycloeucalenol–obtusifoliol isomerase; Fig. 2. NMR spectra (90 MHz) of (a): a sample containing authentic obtusifoliol (**4**) (1.3 mg) and 1.2 mol equivalent

Abstract—Incubation of cycloeucalenol with microsomes of *Zea mays* embryos in ^2H_2O yields obtusifoliol-[19–^{2}H]. Only one ^{2}H atom is incorporated into obtusifoliol during the enzymatic reaction. This has been demonstrated using NMR spectroscopy by correlation of the enzymatically obtained product with ^{2}HCl isomerization products of cycloeucalenol.

INTRODUCTION

9β,19-Cyclopropyl sterols, cycloartenol (**1**), 24-methylene cycloartanol (**2**), and cycloeucalenol (**3**) are considered to be intermediates of sterol biosynthesis in photosynthetic eucaryotes [1, 2]. As phytosterols do not contain a cyclopropane ring, there must be an enzyme that catalyses the opening of the cyclopropane ring of these precursor sterols. Such an enzyme has been discovered in our laboratory [3, 4]. The cyclopropane ring opens when **3** is incubated in the presence of higher plant microsomes [4] yielding obtusifoliol (**4**) another presumed intermediate in phytosterol biosynthesis [1]. Under the same conditions, **1** and **2** are not converted into lanosterol (**5**) and 24-methylene lanostenol (**6**) respectively. These findings are in agreement with a linear biosynthetic scheme proposed by the Liverpool school [5]. In order to increase our knowledge of the reaction mechanism involved during the enzymatic cyclopropane opening reaction, we have performed the isomerization reaction in ^2H_2O. Thus information was obtained concerning the number and position of ^{2}H atoms incorporated into the product of the reaction. These results are compared with those obtained following chemical opening of the 9β,19-cyclopropane ring with ^{2}HCl [6]. A short preliminary report on part of this work has been published [7].

RESULTS

^{2}HCl cleavage of the cyclopropane ring of 24(28)-dihydrocycloeucalenol

^{2}HCl was used to isomerize 24(28)-dihydrocycloeucalenol (**7**). HCl isomerization of 9β,19-cyclo-4,4-dimethyl sterols yields a mixture of $\Delta^{9(11)}$, Δ^8, and Δ^7 isomers [7]. According to the procedure for separating these isomers, described [8, 9] in the case of 4,4-dimethyl-9β,19-cyclosteroid isomerization, the three products obtained following ^{2}HCl isomerization of **7** were 60% of 4α,14α-dimethyl-5α-ergosta-9(11)-en-3β-ol (**8**); 30% of 4α,14α-dimethyl-5α-ergosta-7-en-3β-ol (**9**); and 10% of 24(28)-dihydroobtusifoliol (**10**). GC–MS of the acetates of **8**, **9** and **10** (Table 1) showed a mixture of M + 1, M + 2, and M + 3 molecular ions in all three. However, for the $\Delta^{9(11)}$ isomer, the predominant ion was M + 1 (59%), with M + 2 much less (7%) and M + 3 negligible; for the Δ^7 and Δ^8 isomers, the major molecular ion was M + 2 (45–47%). Such results are fully in agreement with previous findings [10] about the mechanism of acidic isomerization of the 9β,19-cyclopropane ring of cyclosterols. NMR (250 MHz) spectra of the epoxy acetates of **8**, **9**, and **10** showed in each case an incorporation of 0.6 to 1.0 ^{2}H at C-19 (see Experimental).

Table 1. GC–MS determinations of ^{2}H incorporation in the products of ^{2}HCl isomerization of 24(28)-dihydrocycloeucalenol (**7**)

m/e	470 M^+	471 $M^+ + 1$	472 $M^+ + 2$	473 $M^+ + 3$	474 $M^+ + 4$
Acetate of **8**	29*	59	7	4	—
Acetate of **9**	14	19	45	17	4
Acetate of **10**	9	20	47	16	5

* Calculated percentage of each molecular ion species. Results are expressed by taking into account the natural abundance of ^{13}C.

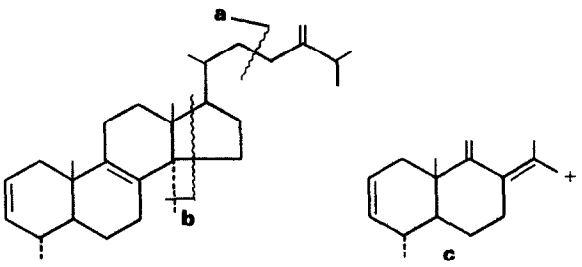
Enzymatic cleavage of the cyclopropane ring of cycloeucalenol (**3**)

Microsomes were incubated (30°; 12 hr) in the presence of cycloeucalenol (150 μM; 1.34 mg) emulsified with Tween 80. Extraction of sterols and chromatography yielded a 4α-methyl sterol fraction, whose constituents were analysed by GC–MS as their TMSi ethers. In these conditions, the substrate (**3**) was unambiguously separated from the product, which was identified as obtusifoliol (**4**). No trace of 4α,14α-dimethyl-5α-ergosta-9(11),24(28)-diene-3β-ol or 4α,14α-dimethyl-5α-ergosta-7,24(28)-diene-3β-ol was detected. The average yield of more than 70% for the conversion of **3** into **4** was reproducible. The

Table 2. MS determination of ^2H incorporation in TMSi ether of enzymatically formed obtusifoliol (4) and in fragments (average of three experiments)

m/e	499 $(M+1)^+$	484 $(M+1)^+-15$	394 $(M+1)^{+\cdot}$ $Si(Me_3OH-15$	310 $(a+1)^+$	228 $(b+1)^+$	215 $(c+1)^+$
	66*	65	60	59	55	55

* Calculated percentage of ion species containing one ^2H. Results are expressed by taking into account the natural abundance of ^{13}C.



MS (Table 2) showed that more than 66% of the molecular ions contained one ^2H (M + 1). Less than 1% of the molecular ions contained two ^2H (M + 2). No incorporated ^2H was detected in unreacted 3. As shown in Table 2, the ^2H labels were retained in fragments a, b, and c [11], in agreement with the hypothesis that ^2H is located at C-19.

Cycloartenol (1) was incubated with microsomes from maize embryos in ^2H_2O. As expected [4], 1 was not converted into 5. Moreover 1 did not incorporate ^2H, as was found above in recovered 3 during conversion of 3 into 4.

NMR spectra of the enzymatically formed obtusifoliol (4)

To determine the precise location of the incorporated ^2H, the NMR (90 MHz) spectra of the mixture (1.1 mg) of cycloeucalenol (3) (30%) and obtusifoliol (4) (70%) from two incubations were recorded in the presence of increasing amounts (0.0:1.4 molar ratio) of the shift reagent, tris(1,1,1,2,2,3,3-heptafluoro-7,7-dimethyl-octane-4,6-diene) Europium $Eu(FOD)_3$. NMR spectroscopic examination of the distribution of ^2H in the C-19 methyl of the biosynthesized obtusifoliol was unsuccessful in the absence of $Eu(FOD)_3$ because of the overlapping of proton signals from the different methyls. But when $Eu(FOD)_3$ was used, all the methyl signals of normal obtusifoliol and cycloeucalenol in $CDCl_3$ were shifted linearly with the amount of $Eu(FOD)_3$ up to 0.7–0.8 mole equivalent (Fig. 1). As the shift was shown to be dependent on the concentration of the sterol (rather low in our case), identical concentrations of sterols were used for the determination of the chemical shift curve and the assay. At *ca* 1:1 molar ratio, the 4α- and 10-methyl resonances were strongly shifted downfield [12–14] and clearly separated from other signals. As shown in Fig 2, comparison of the spectra of pure obtusifoliol (4) and of the deuteriated product showed a decrease in the height and a broadening of the 10-methyl signals in the deuteriated product. The broadening was caused by differences in the chemical shift for CH_3 and CH_2D, and by the CH_2D triplet [15]. Moreover, integration indicated an incorporation of 0.6 ± 0.1 ^2H, using the 4α-methyl resonance of obtusifoliol as internal standard.

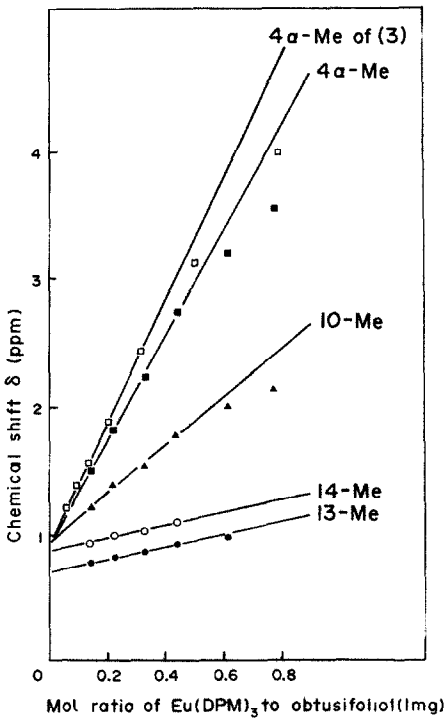


Fig. 1. Variation in the chemical shift for the methyl protons of obtusifoliol in C^2HCl_3 with increasing concentration of $Eu(FOD)_3$.

Correlation of enzymatically produced obtusifoliol (4) *with authentic 24(28)-dihydroobtusifoliol-[19-^2H]* (10)

Because cycloeucalenol (3) and obtusifoliol (4) had been separated as their epoxy acetates [16], we decided to record NMR spectra (250 MHz) of the diepoxy acetate of enzymatically deuteriated 4. The obtained data were correlated with data obtained from authentic diepoxy acetate of unlabelled 4; from the epoxy acetate of 24(28)-dihydro obtusifoliol-[19-^2H] (10) obtained by ^2HCl treatment of 24(28)-dihydrocycloeucalenol (7), acetylation and epoxydation; and finally from authentic epoxy acetate of (10). As shown in Fig 3, comparison of spectra (a) and (b) (^2HCl cleavage) on the one hand and of spectra (c) and (d) (enzymatic cleavage) on the other hand showed the following: (1) The 10-methyl was shifted downfield and clearly separated from the other signals. (2) In both cases, ^2H was incorporated in the 10-methyl, as indicated by lowering and broadening of the C-19 signal in spectra (b) and (d). Integration showed that 0.6 ^2H was incorporated at C-19 in (4) (spectrum d), in excellent agreement with the data obtained above with the shift reagent method.

DISCUSSION

Enzymatic opening, and HCl cleavage of the cyclopropane ring of cycloeucalenol (3) are different by many aspects. When 3 was incubated in the presence of microsomes from corn or bramble cells [4], only obtusifoliol (4) was formed, there being no trace of 4α,14α-dimethyl-5α-ergosta-9(11),24(28)-dien-3β-ol or 4α,14α-dimethyl-5α-ergosta-7,24(28)-dien-3β-ol. When the enzymatic reaction was performed in the presence of ^2H_2O,

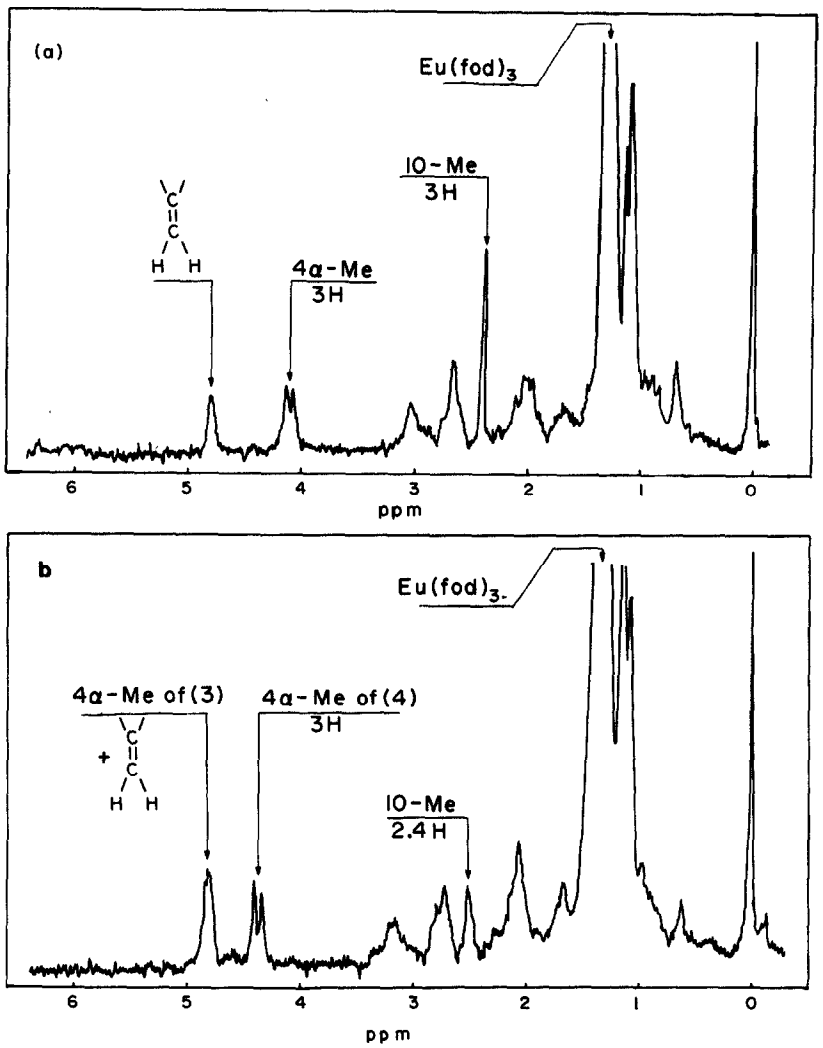


Fig. 2. NMR spectra (90 MHz) of (a): a sample containing authentic obtusifoliol (**4**) (1.3 mg) and 1.2 mol equivalent of $Eu(FOD)_3$, and of (b): a mixture of cycloeucalenol (**3**) (0.5 mg) and biosynthetically deuteriated **4** (1.2 mg) containing 1.4 mol. equivalent of $Eu(FOD)_3$.

one ^2H atom was incorporated into **4**, as shown by GC–MS measurements. 90 MHz NMR of biosynthetically formed **4** in the presence of the shift reagent $Eu(FOD)_3$ showed unambiguously that the ^2H atom was incorporated at C-19, and 250 MHz NMR of the diepoxyacetate of enzymatically formed **4** entirely confirmed this result. When **7** was treated with HCl, the $\Delta^{9(11)}$ isomer (**8**) was the main product (60%); the Δ^7 isomer (**9**) was also formed but to a lesser extent (30%), while 24-dihydroobtusifoliol was a minor product (less than 10%). When this reaction was performed with ^2HCl, MS and NMR showed that whereas one ^2H was incorporated at C-19 into the $\Delta^{9(11)}$ isomer, two ^2H atoms were incorporated into the major ion species (M + 2) of both the Δ^7 (**9**) and Δ^8 (**10**) isomers, one ^2H atoms occurring at C-19 and the other probably at C-7 or C-9, reflecting the existence of an equilibrium between **9** and **10**, as suggested previously [10]. The following conclusions may be drawn from these results. Firstly the enzymatic reaction could be considered as a biochemical step equivalent to an acid catalysed cyclopropane ring opening reaction. Secondly the production of obtusifoliol (**4**) in the enzymatic system does not involve passage through a $\Delta^{9(11)}$ intermediate, as shown by the absence of such an intermediate from the reaction products and the absence in **4** of ^2H incorporation in any other position than C-19. Intermediacy of a $\Delta^{9(11)}$ product could be envisaged for the enzymatic formation of **4**; formation of **4** involves 9β-19-bond cleavage of the cyclopropane ring and 8β-H elimination. As 9β,19-H bonds are *cis*, a concerted mechanism is not likely, whereas passage through a $\Delta^{9(11)}$ intermediate followed by a Δ^9 to a Δ^8 isomerization, involving *trans* eliminations of the 11α-H and 8β-H, constitutes a reasonable possibility. However this interpretation is definitely ruled out by our results. Finally our results would be in agreement with a previously proposed mechanism [17] involving an intermediate **11** (Scheme 1) in which the C-9 carbonium ion is stabilized (ionic pair or covalent bond) by a suitable enzymatic group; in these conditions obtusifoliol (**4**) is obtained via a *trans*-antiperiplanar elimination of the 8β-H from **11**.

No ^2H was shown to be incorporated into **3** during its

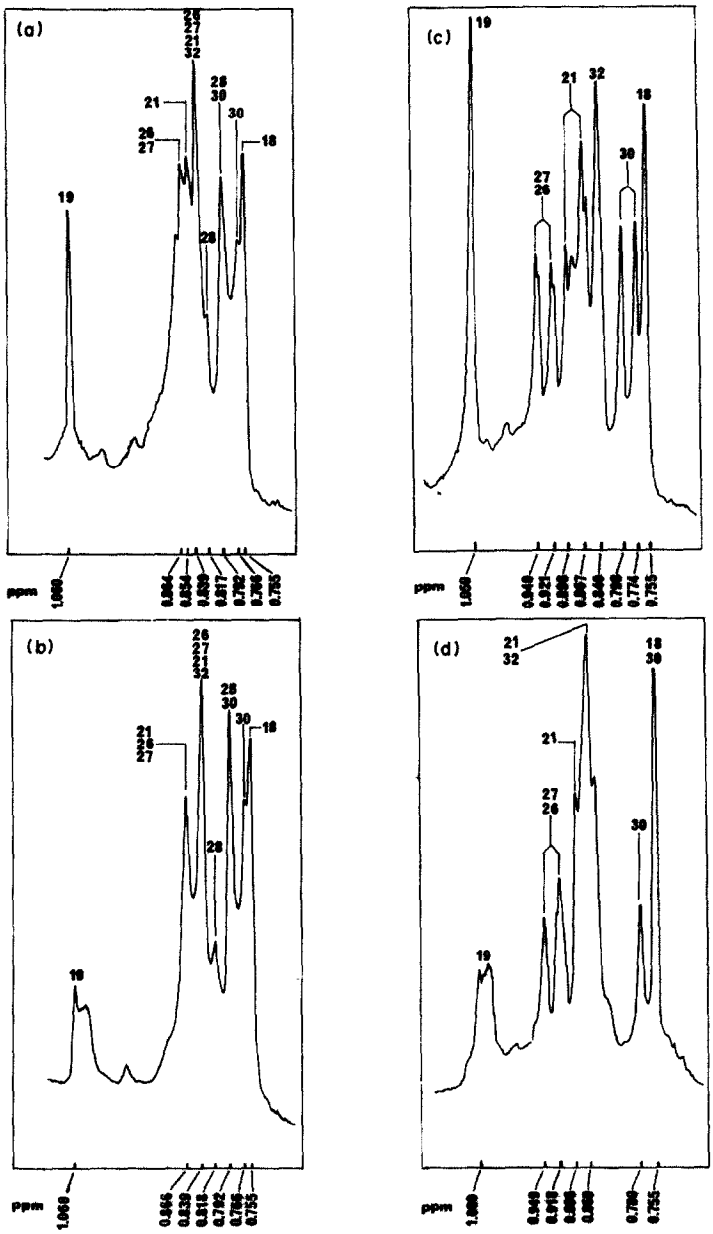
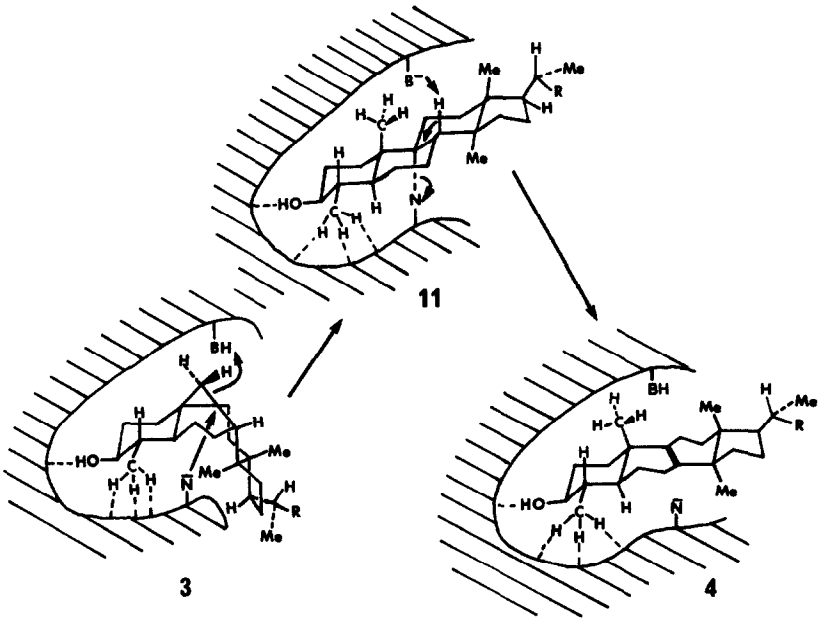


Fig 3 NMR spectra (250 MHz) of (a) epoxyacetate of 24(28)-dihydroobtusifoliol (**10**); (b) epoxyacetate of 24(28)-dihydroobtusifoliol-[19-^{2}H]. (c) diepoxy acetate of obtusifoliol (**4**); (d) diepoxyacetate of enzymatically deuterated obtusifoliol (**4**).

conversion into **4**; moreover only one ^{2}H was shown to be incorporated at C-19 in **4**. It follows that the transformation of **3** into the postulated intermediate **11** proceeds without exchange of the 19-proton with protons from the medium, and so this latter step could be considered irreversible. This interpretation, however, implies that the 10-methyl group in **11** retained its free rotation. The irreversibility of this step could reflect the boat-to-chair conformation change of the B cycle during conversion of **3** into **11**. Cycloartenol (**1**) was incubated with microsomes from maize embryos in ^2H_2O. As expected [4], **1** was not converted into lanosterol. Moreover **1** had not incorporated ^{2}H suggesting that when **1** is incubated, the cyclopropane ring cleavage rather than the elimination of 8β-H from **11** could be the blocked step. It has been suggested [4] that the presence of a 4β-methyl in **1** could hinder the action of the enzyme in some way, either by interfering with the binding of enzyme and substrate or



by hindering the approach of a chemical group essential for the opening of the cyclopropane ring.

EXPERIMENTAL

TLC was carried out on Merck HF 254 plates (0.2 mm). For argentation TLC plates were immersed in 10% soln $AgNO_3$ in EtOH–H_2O (3:1), dried for 12 hr at room temp., and activated for 30 min at 110°. After spraying with 0.1% soln of berberin hydrochloride in EtOH, the products were observed under UV (340 nm). GLC employed a GC fitted with two FID and two glass columns (1.50 m × 3 mm) packed with either 1% SE-30 or 1% OV-17. GC–MS was carried out at an ionizing energy of 70 eV. The separation was carried out at 270° on a glass column packed with 1% Dexsil. NMR spectra were in $CDCl_3$; the chemical shifts of signals are given in δ with TMS as the internal standard.

Authentic materials. Cycloeucalenol was extracted [4] from eucalyptus wood (*Eucalyptus robusta*) kindly supplied by Dr. E. B. Huddlerton (Sydney, Australia) > 99% GLC purity, (RR_t to cholesterol: 1.80), mp 139–140°, (lit. [18] 140°). Obtusifoliol was extracted [4] from the latex of *Euphorbia obtusifolia*, which was kindly supplied by Prof. A. Gonzales Gonzales (Las Palmas, Las Canarias, Spain). Cycloartenol was kindly supplied by Prof. G. Ourisson. 24(28)-dihydrocycloeucalenol (**7**) was prepared by hydrogenating cycloeucalenol (76 mg) in EtOAc (5 ml) in the presence of PtO_2 (30 mg) for 2 hr at room temp. After removal of the catalyst, (**7**) (76 mg) was recovered: > 99% GLC purity (SE 30), *m/e* 428, 413, 395, 302, 301, 175. ^{2}HCl was supplied by the Commissariat à l'Energie Atomique (Gif sur Yvette, France).

^{2}HCl isomerization of **7**. A soln of **7** (70 mg) in C^2HCl_3 (3 ml) was treated with a vigorous stream of dry ^{2}HCl at room temp. for 30 min. The C^2HCl_3 was evaporated and after drying under red. pres. the residue was acetylated at room temp. for 14 hr using a mixture of C_5H_5N (500 µl) and Ac_2O (1 ml). Excess of reagents was evapd. under red. pres. and the crude acetates (63 mg) purified on PLC using CH_2Cl_2. Analytical argentation TLC, using cyclohexane–C_6H_6 (3:1) as developing solvent, 15 hr of migration, of the isomerized product gave 2 bands separated into 3 fraction. The band closest to the starting line (fraction 1) corresponded to the acetate of 4α,14α-dimethyl-5α-ergosta-9(11)-en-3β-ol (**8**) (29 mg): > 99% GLC purity, (RR_t to **7**: 0.96, OV-17); mp 117.5–118.5° from MeOH, IR: $\nu_{max}^{CHCl_3}$, cm^{-1}: 1730, 1380, 1365 and 1260; GC–MS (HCl isomerization) *m/e*: 470, 455, 395, 301, 287, 241, 227, 215. (^{2}HCl isomerization) *m/e*: 470 (29), 471 (59), 472 (7), 473 (4), 474 (0), 456, 396, 302, 288, 242, 228, 216. The band closest to the solvent front gave fractions 2 and 3. Fraction 2 (19 mg) corresponded to the $\frac{2}{3}$ of the band closest to the starting line. It contained the acetates of 4α,14α-dimethyl-5α-ergosta-7-en-3β-ol (**9**) (80%) and of 24(28)-dihydroobtusifoliol (**10**) (20%). Fraction 3 (6.5 mg) corresponded to the remaining $\frac{1}{3}$ of this band closest to the solvent front. It contained a mixture of the acetates of **9** (35%) and **10** (65%). Fractions 2 and 3 were rechromatographed separately on argentated TLC, yielding acetates of **9** and **10**. Acetate of **9** (14 mg): 85% GLC purity, (RR_t to **7**: 1.00, OV-17); GC–MS (HCl isomerization) *m/e*: 470, 455, 395, 301, 287, 256, 241, 227, 215. (^{2}HCl isomerization) *m/e*: 470 (14), 471 (19), 472 (45)[1] 473 (17), 474 (4), 457, 397, 303, 289, 258, 243, 229, 217. Acetate of **10** (2.5 mg) 90% GLC purity, (RR_t to **7**: 0.81, OV-17); GC–MS (HCl isomerization): *m/e* 470, 455, 395, 301, 287, 241, 227, 215; (^{2}HCl isomerization) *m/e*: 470 (9), 471 (20), 472 (47), 473 (16), 474 (5), 457, 397, 303, 289, 243, 229, 217. The acetates of **8**, **9**, and **10** from ^{2}HCl isomerization were epoxidized with *p*-nitroperbenzoic acid (40 mg) in dry Et_2O (1 ml) as described earlier [16]. Epoxy-acetates were chromatographed on TLC using CH_2Cl_2 as developing solvent, giving pure epoxy-acetates. 4α,14α-Dimethyl-5α-ergosta-9(11)-epoxy-3β-yl-[19-^{2}H] acetate (7 mg) mp 161–163° from MeOH; NMR (250 MHz, CCl_4): δ 0.758 (3H, *s*; C-18), 0.771 (3H, *d*, *J* = 6.5 Hz, C-4α), 0.802 (3H, *d*, *J* = 5.3 Hz, C-28), 0.838 (3H, *s*, C-14), 0.846 (6H, *d*, *J* = 3.8 Hz; C-26, -27), 0.859 (3H, *d*, *J* = 3.3 Hz, C-21), 1.136 (2.3H, *s*, C-19). 4α,14α-Dimethyl-5α-ergosta-7-epoxy-3β-yl-[19-^{2}H] acetate (8 mg): NMR (250 MHz, CCl_4): δ 0.774 (3H, *d*, *J* = 7 Hz, C-4α), 0.788 (3H, *s*, C-18), 0.804 (3H, *d*, *J* = 7.7 Hz, C-28), 0.856 (9H, *d*, *J* = 6.3 Hz; C-26, -27, C-21), 0.890 (2.2H, *s*, C-19), 0.981 (3H, *s*, C-14). 4α,14α-Dimethyl-5α-ergosta-8-epoxy-3β-yl-[19-^{2}H] acetate (2 mg): NMR (250 MHz, CCl_4) (Fig. 3): δ 0.755 (3H, *s*, C-18), 0.779 (3H, *d*, *J* = 6.5 Hz, C-4α), 0.805 (3H, *d*, *J* = 6.5 Hz, C-28), 0.839 (3H, *s*, C-14), 0.852 (9H, *d*, *J* = 6.7 Hz; C-26, -27, C-21), 1.060 (2.1H, *s*, C-19). 8,24(28)-Diepoxy acetate of obusifoliol (**4**) and 8-epoxy acetate of 24(28)-dihydro obtusifoliol (**10**) were prepared from authentic obtusifoliol and NMR spectra

recorded (Fig 3). 8,24(28)-Diepoxy acetate of **4**: NMR (250 MHz, CCl_4): δ 0.755 (3H, *s*, C-18), 0.786 (3H, *d*, J = 6.0 Hz, C-4α), 0.840 (3H, *s*, C-14), 0.840 (3H, *s*, C-14), 0.882 (3H, *d*, J = 7.5 Hz, C-21), 0.935 (6H, *d*, J = 6.7 Hz, C-26, -27), 1.059 (3H, *s*, C-19). 8-epoxy acetate of **10**: NMR (250 MHz, CCl_4): δ 0.755 (3H, *s*, C-18), 0.779 (3H, *d*, J = 6.5 Hz, C-4α), 0.804 (3H, *d*, J = 6.5 Hz, C-28), 0.839 (3H, *s*, C-14), 0.847 (3H, *d*, J = 3.8 Hz C-21), 0.851 (6H, *d*, J = 6.3 Hz, C-26, -27), 1.060 (3H, *s*, C-19).

Preparation of subcellular fractions. Corn seeds (*Zea mays*, var. Inra 258) were soaked for 5 hr in H_2O before being planted in moist vermiculite. They were grown for 60 hr at 25°. The embryos (70 g) were ground in a mortar at 0° with 3 vol. of medium containing: 0.1 M Tris–HCl, 0.3% BSA, 10 mM mercaptoethanol, 0.5 M sucrose, 4 mM $MgCl_2$, final pH 7.5. The homogenate was squeezed through two layers of cheese cloth and centrifuged at 2000 *g* for 6 min. The supernatant was centrifuged at 105000 *g* for 1 hr. Soluble supernatant was removed and the microsomal pellets were suspended in 15 ml of ^2H_2O medium containing: 0.1 M phosphate buffer pH 8.3, 5 mM mercaptoethanol, 2 mM $MgCl_2$, and dispersed in a Potter–Elvehjem homogenizer. The homogenate was centrifuged at 105000 *g* for 1 hr and the pellet resuspended in 17.5 ml of the same medium as before; the final pH was 8.

Enzymatic assays. The dispersed microsomal pellet (17.5 ml) was incubated in the presence of cycloeucalenol (3.15 μmol = 1.34 mg) dissolved in 3.5 ml 0.6% Tween-80 in ^2H_2O resuspension medium for 12 hr at 31°. The reaction was terminated by the addition of 20% KOH in EtOH (1 vol.). Cycloartenol incubations were carried out in the same way.

Analytical procedure. The incubation mixture was extracted 3× with petrol (50 ml). Combined extracts were dried over Na_2SO_4, evapd. under red. pres. and separated by TLC with CH_2Cl_2 as the solvent (2 runs). The band of 4α-methyl sterols was scraped off and eluted. The 4α-methyl sterols were analysed by GC–MS as their TMSi ethers. The substrate (30%) was unambiguously separated from the product (70%) of the reaction and MS were recorded. TMSi ether of (**4**): GC–MS, *m/e* (rel. int.): 499 (19), 498 (7.5), 484 (42), 483 (18), 394 (42), 393 (23.5), 228 (13), 227 (8.5), 216 (12), 215 (8), 190 (5), 189 (6). In the case of cycloartenol incubations, the same analytical procedure was employed except that the 4,4-dimethyl sterol band was scraped off the plate. The NMR (90 MHz) spectra of a mixture (1.7 mg) of **3** (30%) and **4** (70%) coming from several incubations were recorded in the presence of increasing amounts (0.0–1.4 molar ratio) of $Eu(FOD)_3$. After TLC separation using EtOAc–cyclohexane (1:9, two migrations) as developing solvent, acetylation and epoxydation, performed as described above, gave the following results: 24(28)-epoxy acetate of **3** and 8,24(28)-diepoxy acetate of **4** (0.5 mg), the NMR spectrum of which was recorded (Fig 2): δ 0.755 (3H, *s*, C-18), 0.768 (3H, *d*, J = 6.5 Hz, C-4α), 0.869 (3H, *s*, C-14), 0.882 (6H, *d*, J = 6.8 Hz, C-21), 0.935 (6H, *d*, J = 7.5 Hz, C-26, -27) 1.060 (2.4H, *s*, C-19).

Nomenclature. Cycloartenol = 4,4,14α-trimethyl-9β,19-cyclo-5α-cholest-24-en-3β-ol (**1**); 24-methylene cycloartenol = 4,4,14α-trimethyl-9β,19-cyclo-5α-ergost-24(28)-en-3β-ol (**2**); Cycloeucalenol = 4,14α-dimethyl-9β,19-cyclo-5α-ergost-24(28)-en-3β-ol (**3**); Obtusifoliol = 4,14α-dimethyl-5α-ergosta-8,24(28)-dien-3β-ol (**4**); Lanosterol = 4,4,14α-trimethyl-5α-cholesta-8,24-dien-3β-ol (**5**); 24-methylene lanostenol = 4,4,14α-trimethyl-5α-ergosta-8,24(28)-dien-3β-ol (**6**); 24(28)dihydrocycloeucalenol = 4,14α-dimethyl-9β,19-cyclo-5α-ergostane-3β-ol (**7**); 4,14α-dimethyl-5α-ergost-9(11)-en-3β-ol (**8**); 4,14α-dimethyl-5α-ergost-7-en-3β-ol (**9**); 24(28)-dihydroobtusifoliol = 4,14α-dimethyl-5α-ergost-8-en-3β-ol (**10**).

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REFERENCES

1. Goad, L. J. and Goodwin, T. W. (1972) in *Progress in Phytochemistry* (Rheinhold, L. and Liwschitz, Y., eds.) Vol III, p. 113. Interscience, London.
2. Benveniste, P., Hirth, L. and Ourisson, G. (1966) *Phytochemistry* **5**, 45.
3. Heintz, R., Benveniste, P. and Bimpson, T. (1972) *Biochem. Biophys. Res. Commun.* **49**, 820.
4. Heintz, R. and Benveniste, P. (1974) *J. Biol. Chem.* **249**, 4267.
5. Goad, L. J. and Goodwin, T. W. (1966) *Biochem. J.* **99**, 735.
6. Bentley, H. R., Henry, J. A., Irvine, D. S. and Spring, F. S. (1953) *J. Chem. Soc.* 3673.
7. Rahier, A., Benveniste, P. and Cattel, L. (1976) *J. Chem. Soc., Chem. Comm.* 287.
8. Rees, H. H., Goad, L. J. and Goodwin, T. W. (1968) *Biochem. J* **107**, 417.
9. Itoh, T., Tamura, T. and Matsumoto, T. (1975) *Lipids* **10**, 454.
10. Ourisson, G., Crabbe, P. and Rodig, O. R. (1964) *Tetracyclic Triterpenes*, p. 51. Hermann, Leeds.
11. Budzikiewicz, H., Wilson, J. M. and Djerassi, C. (1963) *J. Am. Chem. Soc.* **85**, 3688.
12. Rondeau, R. E. and Sievers, R. E. (1971) *J. Am. Chem. Soc.* **93**, 1522.
13. Kocor, M. and Pyrek, J. St. (1973) *J. Org. Chem.* **38**, 3688.
14. Suga, T. and Shishibori, T. (1975) *Phytochemistry* **14**, 2411.
15. MacDonald, C. G., Shannon, J. S., and Sternhell, S. (1964) *Australian J. Chem.* **17**, 38.
16. Ponsinet, G. and Ourisson, G. (1968) *Phytochemistry* **7**, 757.
17. Goad, L. J., Williams, B. L. and Goodwin, T. W. (1967) *European J. Biochem.* **3**, 232.
18. Cox, J. S. G., King, F. E. and King, T. J. (1956) *J. Chem. Soc.* 1384.