Antileukemic Pseudoguaianolides from *Hymenoxys grandiflora* (T. & G.) Parker. Application of Lanthanide-Induced Shifts to Structure Determination[1,2]

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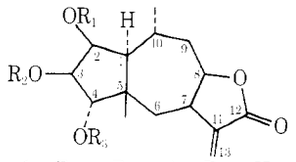
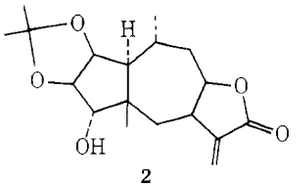
Hymenoxys grandiflora (T. & G.) Parker yielded three new pseudoguaianolides, hymenograndin, florigrandin, and hymenoflorin, and the previously known pseudoguaianolide glucoside paucin. Structures and stereochemistry of the new compounds were established by a combination of chemical transformations and physical methods. In particular, the stereochemistry of hymenograndin at C-4 was deduced by interpreting lanthanide-induced shifts using the modified McConnell equation. Structure determination of hymenoflorin and florigrandin which were correlated required nmr spectrometry at 270 MHz. Hymenoflorin exhibited significant *in vivo* activity against L-1210 lymphocytic leukemia, paucin against P-388 leukemia.

The genus *Hymenoxys* is rich in sesquiterpene lactones of the pseudoguaianolide and modified pseudoguaianolide type.[3-5] In the present communication we report the isolation and structure determination of three new pseudoguaianolides, **1a**, **5a**, and **6a**, which we have named hymenograndin, florigrandin, and hymenoflorin, from *Hymenoxys grandiflora* (T. & G.) Parker (old-man-of-the-mountain). This is a previously uninvestigated species which enjoys a brief flowering period in the alpine tundra of the Rocky Mountains during July and early August. The known[3,4,6] pseudoguaianolide glucoside paucin (**11**) was also found.[7]

Hymenograndin, $C_{19}H_{26}O_7$, mp 153–154°, $[\alpha]D$ +80.7°, the least polar constituent, had a tendency to form solvates, which complicated determination of the empirical formula and initially interfered with interpretation of the nmr spectrum. It was a diacetate (high-resolution mass spectrum, two three-proton resonances at 2.08 and 2.03 ppm) and had a free hydroxyl group (ir spectrum, conversion to a triacetate **1b**). The nmr spectrum also exhibited the typical doublets of an exocyclic methylene group conjugated with a lactone function (H-13a and H-13b of formula **1**), a multiplet near 4.8 ppm, presumably the signal of hydrogen under the lactone ether oxygen which remained stationary during acetylation while a doublet originally at 3.62 ppm (hydrogen under a secondary hydroxyl group) moved downfield into a two-proton cluster in the range 4.8–5.1 ppm (hydrogens under the acetates, assignment confirmed by hydrolysis to **1c** which resulted in the expected upfield shift). Since the two esterified secondary hydroxyl groups, one free secondary hydroxyl group, and the lactone function accounted for all the oxygen atoms of the empirical formula, the absence of additional double bonds and the presence, in the nmr spectrum, of a methyl singlet at 0.97 ppm and a methyl doublet at 1.08 ppm indicated that hymenograndin was an eudesmanolide or a pseudoguaianolide.



1a, $R_1 = R_2 = Ac$; $R_3 = H$
b, $R_1 = R_2 = R_3 = Ac$
c, $R_1 = R_2 = R_3 = H$



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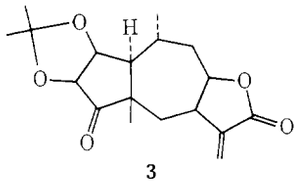

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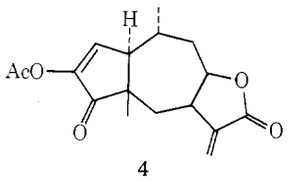

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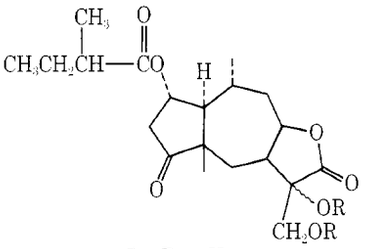

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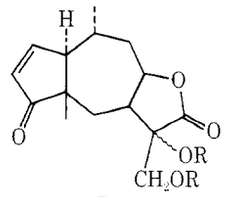
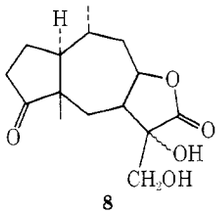
b, R = Ac



6a, R = H
b, R = Ac
c, $R = B_2$



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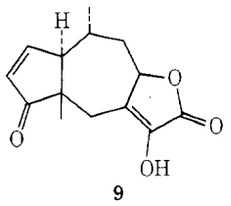

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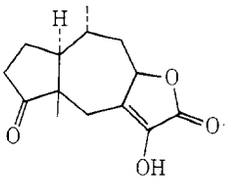

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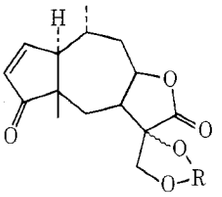
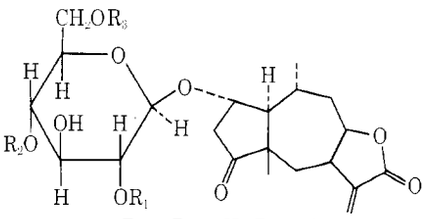


7a, R = >CHCH$_3$
b, R = O=S



11, $R_1 = R_2 = H$; $R_3 = Ac$
or $R_1 = R_3 = H$; $R_2 = Ac$
or $R_2 = R_3 = H$; $R_1 = Ac$

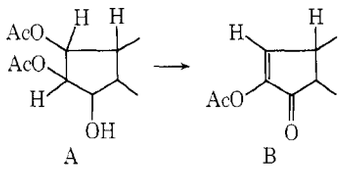
Acid hydrolysis of hymenograndin in aqueous acetone or treatment of **1c** with acetone–toluenesulfonic acid afforded an acetonide **2** whose nmr spectrum (see Experimental Section) indicated that only the newly freed hydroxyl groups but not the hydroxyl group originally present in

Table I
Nmr Spectrum of 2[a]

H-1	6.79	$J_{1,10} = 11.5$
H-2	9.93	$J_{1,2} = 6.6$
H-3	15.93	$J_{2,3} = 8.0$
H-4	14.68	$J_{3,4} = 4.9$
H-6α	6.79	$J_{6\alpha,6\beta} = 14 \pm 0.5$, $J_{6\alpha,7} = 4 \pm 0.5$
H-6β	4.26	$J_{6\beta,7} = 15.5 \pm 0.5$
H-7	5.17	$J_{7,13a} = 2.4$, $J_{7,13b} = 2.2$
H-8	6.67	$J_{7,8} = 8 \pm 0.5$
H-9α	4.18	$J_{8,9\alpha} = 4 \pm 0.5$, $J_{9\alpha,9\beta} = 13.4 \pm 0.5$
H9β	[b]	$J_{8,9\beta} = 11 \pm 0.5$
H-10	4.82	$J_{9\beta,10} \leq 0.7$, $J_{9\beta,10} \cong 6$
H-13a	4.06	$J_{10,14} = 6.6$
H-13b	6.42	$J_{13a,13b} \leq 0.2$
H-14[c]	3.43	
H-15[c]	5.40	
Acetonide methyls[c]	5.91, 7.31	
OH	23.7	

[a] Run at 90 MHz in $CDCl_3$ with TMS as internal standard at $Eu(DPM)_3$ concentrations of 0, 0.16, 0.36, 0.41, 0.80, and 0.95 mol/mol of **2**. Chemical shifts are those observed in the 0.95 *M* solution; coupling constants (hertz) were determined by direct observation or double irradiation in whatever solution gave the best separation of the signals being observed. [b] Not determined. [c] Three protons.

hymenograndin had participated in acetal formation. Oxidation of **2** resulted in genesis of a cyclopentanone **3** (lactone and ketone bonds superimposed at 1760 cm^{-1}); the accompanying downfield shifts of the ether signals and their appearance (AB system in which B but not A was coupled to a third proton C) suggested that formation of the acetonide involved oxygens α and β to the new carbonyl function, *i.e.*, that hymenograndin possessed partial structure A where the acetate functions must be cis. Confirmation for this inference was provided by the transformation of **1a** with chromic acid to an α-acetoxy-α,β-unsaturated cyclopentenone of type B (λ_{max} 240 nm, new infrared frequencies at 1720 and 1610 cm^{-1}, replacement of the two-proton cluster of A near 5 ppm by a one-proton doublet at 7.00 ppm) as the result of β-elimination of acetic acid.



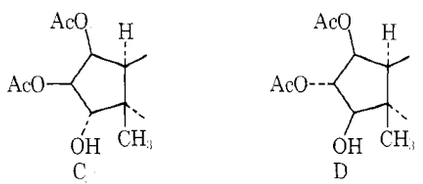
The complete structural formula of hymenograndin was deduced by extensive spin-decoupling studies on the acetonide **2** at various concentrations of the lanthanide shift reagent $Eu(DPM)_3$.[8] The results, presented in Table I, were obtained in the usual way; *i.e.*, irradiation at the frequencies of H-13a and H-13b permitted identification of H-7 and irradiation at the frequency of H-7 established the presence of an adjoining methylene group, neither one of whose protonic components (rendered visible at higher concentrations of shift reagent) was coupled to other protons, and established the remaining vicinal proton as the proton under the lactone ether oxygen (H-8). Irradiation at the frequency of the latter not only collapsed the H-7 signal, but established the presence of neighboring H-9α and H-9β. The chemical shift of H-10, close to that of H-9α and H-9β at low concentrations of shift reagent, was established by irradiation at the frequency of the methyl doublet; observation of H-10 and one of the H-9 protons (H-9α) at high concentrations of shift reagent permitted determination, by irradiation at the frequency of H-8, of the values of $J_{8,9\alpha}$, $J_{9\alpha,10}$, $J_{9\alpha,9\beta}$, and $J_{9\beta,10}$.

Samek's rule[9] that $J_{7,13}$ trans ≥ 3 Hz $\geq J_{7,13}$ cis indicated that the lactone ring of hymenograndin was cis fused; if H-7 is α as in all pseudoguaianolides of authenticated stereochemistry, this is in agreement with the observation of a negative Cotton effect at 255 nm associated with the n,π^* transition of a cis-fused, α,β-unsaturated lactone closed to C-8.[11] Construction of Dreiding models and comparison of the observed coupling constants with those predicted from the dihedral angles of the models led to the conclusion that the observed coupling constants are best satisfied if the seven-membered ring assumes a boat conformation in which the cis-lactone ring is somewhat flexible and if H-1 and C-10 methyl are α, as in all other pseudoguaianolides from *Helenium* and related species.

The remaining problem was the stereochemistry at C-2, C-3, and C-4, which, because only the C-2 and C-3 hydroxyl groups formed an acetonide, had to be either that shown in C or in D. Knowledge of the coupling constants involving H-1, H-2, H-3, and H-4 was not sufficient to decide between these alternatives. However, development of a method based on the quantitative prediction of lanthanide shifts[13] using the modified McConnell equation[14]

$$\left(\frac{\Delta H}{H}\right)_i = \frac{K(3\cos^2\chi_i - 1)}{r_i^3} \quad (1)$$

so as to determine the configuration of a hymenograndin derivative capable of coordinating with the ion appeared to have promise.[15,16] The best for this purpose of the available derivatives of hymenograndin appeared to be **2** because of the expectation that it would form a single coordination complex involving only the alcohol oxygen atom.[17]



To provide a basis for using the method of lanthanide-induced shifts to the determination of the configuration of hymenograndin, we decided to determine initially how well eq 1 correlated with the lanthanide-induced shifts of 26 protons, not subject to contact shift, in four model compounds studied by Demarco and coworkers[18] (their compounds 1–4). These data were also useful for evaluating the model necessary for the computations.

The assumption was made that the complex formed between $Eu(DPM)_3$ and **2** would be similar in geometry and interaction kinetics to the complexes formed between $Eu(DPM)_3$ and the four compounds studied by Demarco, *et al.* It was also assumed that there would be either free rotation about the O–C* bond of the complex or that complexes would be formed between $Eu(DPM)_3$ and all rotational isomers of the alcohol. Since in all instances reported so far chemical exchange has been faster than nmr time, the mathematical treatment of both possibilities would be the same. To allow for easier computation, the rotational capability was treated in terms of two static models, one corresponding to closest approach of the europium atom and the proton in question, the other to the greatest distance between the europium, while still coordinated, and the same proton.

The computer program was written[19] such that the spatial parameters needed were the C*-O-H_i angle, the O-H_i bond distance, and an initial estimate of both the C*-O-Eu angle and the Eu-O distance. Conversion of input data to the two europium positions is accomplished within the program by trigonometric manipulation. Use was made of Dreiding models and other published data[20] to obtain initial estimates of the C*-O-Eu angle (125°) and the Eu-O distance (2.50 Å) in the complexes. These estimates were substituted in eq 1 to calculate individual values of K for the 26 protons, not subject to contact shift, listed by Demarco, *et al.*[18] The standard deviation of an individual result for K was calculated from the set of K's generated using the initial estimates of europium angle and distance. A small increment (0.035 radian and 0.1 Å, respectively) was then added to angle and distance and a new set of K's was calculated. The process was continued on an iterative basis until a minimum standard derivation for K had been reached; further refinement was performed using incremental values of 0.0035 radian and 0.01 Å. This process yielded 1530 as a value for K with a standard deviation of 11% and values of 139° for the angle and 4.19 Å for the distance.

Spectra of **2** were measured at 90 MHz using $CDCl_3$–TMS solutions containing 0, 0.16, 0.36, and 0.41 mol of $Eu(DPM)_3$ per mole of **2**. Since exchange was more rapid than nmr time, the spectra contained only a single time-averaged set of resonances for **2** and its complexed form. Only a limited number of signals could be followed over the range of shift concentrations. These are listed in Table II; assignments were confirmed by double irradiation as discussed earlier.

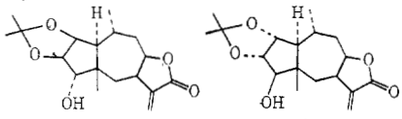
The magnitude of induced shift was measured at each $Eu(DPM)_3$ concentration and a linear least-squares fit for the data was obtained. Following convention, the induced shift was extrapolated to a 1:1 mole ratio of shift reagent compared with shifts calculated by using the values of K, Eu-O distance, and Eu-O-C* angle obtained from the four model compounds and by using C(4)-O-H_i angles and O-H_i's measured from models of **2** (based on C, column 3) and **2′** (based on D, column 5).

While the agreement between calculated and observed shifts for H-3 and H-4 is somewhat less than was hoped for and the differences between the two sets of calculated values are, on the whole, not great, one significant datum emerges immediately on inspection of Table II. The observed shift of the C-5 methyl group, in close proximity to the hydroxyl group on C-4, is reasonably close to that predicted for formula **2**, while vastly different from the value predicted for formula **2′**. Consequently, we feel that there is little doubt that the C-4 hydroxyl group of **2** is α and that hymenograndin is correctly represented by formula **1a**.

Contrary to our previous experience with lanthanide-induced shifts of α,β-unsaturated lactones, the observed shifts of the exo-methylene protons H-13a and H-13b of **2** were upfield, as were the values calculated for these protons. Although the numerical agreement was not particularly good for H-13a, the circumstance that the upfield shifts predicted by the method were in fact observed experimentally lends credibility to the chosen model and to the assumptions that were employed.

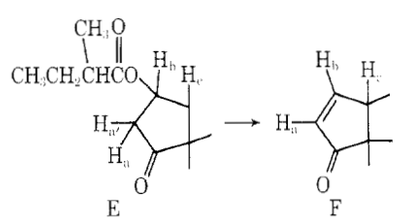
Florigrandin, $C_{20}H_{30}O_7$, mp 173–175°, and hymenoflorin, $C_{15}H_{20}O_5$, mp 197–199°, are conveniently discussed together since treatment of the former with acetone–HCl (or preparative tlc of florigrandin) resulted in conversion to the latter by β-elimination of a saturated five-carbon ester side chain. While florigrandin was a saturated ketone (λ_{max} 282 nm, ϵ 120), hymenoflorin was clearly an α,β-unsaturated cyclopentenone of type F because of its ir bands at 1700 and 1574 cm^{-1}, the uv spectrum (λ_{max} 217.5 nm), and the typical nmr doublets of doublets at 7.76 (β proton) and 6.06 ppm (α proton) which disappeared on catalytic hydrogenation to dihydrohymenoflorin (**8**). In the nmr spectrum of florigrandin these signals were replaced by a multiplet at 5.11 ppm which represented the proton at the point of attachment of the ester side chain, undoubtedly at the β position of the cyclopentanone ring as in E (for confirmation, *vide infra*).

Table II
Lanthanide-Induced Shifts of 2

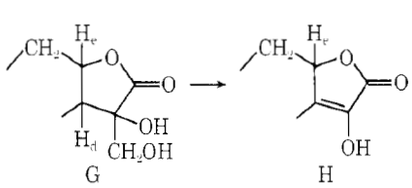


2 2′

Proton	Δ_{obsd}	$\Delta\delta_{calcd}$	Difference	$\Delta\delta_{calcd}$	Difference
H-2	6.4	6.8	0.4	6.8	0.4
H-3	12.8	16.2	3.4	15.6	2.8
H-4	11.7	15.1	3.4	15.7	3.0
C-5 Me	6.4	7.1	0.7	17.0	10.6
C-10 Me	2.7	3.1	0.4	3.4	0.7
H-13a	−1.6	−4.0	2.4		
H-13b	−0.1	−0.7	0.8		
Acetonide	5.1	3.4	−1.7	3.3	−1.8
Me	5.2	3.5	−1.7	4.3	−0.9



E F



G H

The nature of the five carbon ester side chain was not immediately evident from the nmr spectrum of florigrandin, as its signals, regardless of solvent, were superimposed on those of a methyl singlet and a methyl doublet also displayed in the nmr spectra of hymenoflorin and its derivatives. However, use of chemical shift reagents demonstrated that the side chain gave rise to a methyl doublet and a methyl triplet, thus identifying florigrandin as a 2-methylbutanoyl ester of type E.

Florigrandin and hymenoflorin were diols (ir spectra, conversion to diacetates). The nmr spectra revealed an AB quartet near 3.6 ppm which was shifted downfield on transformation to the diacetates **5b** and **6b**. Hence the grouping R_3C-CH_2OH, where $R \neq H$, was present. The absence of other paramagnetic shifts as the result of acetylation indicated that the second hydroxyl group was tertiary and, because of the facility with which it underwent acetylation, α[21] to the γ-lactone group (ir band near 1770 cm^{-1}). Hence a plausible partial structure was G.

Confirmation for the presence of the α-glycol function G was provided by the formation, from hymenoflorin, of an ethylidene derivative **7a** and a thiocarbonate **7b** in whose nmr spectra all signals except those of the $-CH_2OH$ group were essentially unchanged. Moreover, periodate oxida-

tion of hymenoflorin and dihydrohymenoflorin resulted in transformation to norenol lactones of type H. This was accompanied by a paramagnetic shift and simplification of a complex signal, previously found near 4.78 ppm, to a triplet at 5.25 ppm. Hence the lactone ring of florigrandin and hymenoflorin must be closed to C-8 if these substances are pseudoguaianolides like hymenograndin and they can be formulated as **5a** and **6a**, respectively, exclusive of stereochemistry.

This conclusion was confirmed by extensive spin decoupling experiments on **6b** at 90 and 270 MHz which provided the data[22] reproduced in Table III and permitted independent deduction of the carbon skeleton of hymenoflorin and thereby that of florigrandin.

With respect to the stereochemistry, the CD curve of hymenoflorin exhibited the strong negative Cotton effect near 325 nm characteristic of the trans-fused cyclopentenone system and absolute stereochemistry (H-1α, C-5 methyl α) depicted in the formula, an inference supported by the inversion of the Cotton effect which accompanied

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EXPERIMENTAL[25]

Extraction of Hymenoxys grandiflora. -- A) Dried and ground Hymenoxys grandiflora (T. & G.) Parker, wt. 1.1 kg, collected by Dr. B. H. Braun on August 2, 1962 on the tundra off Trail Ridge Drive, Rocky Mountain National Park, with permission of the National Park Service, was extracted with chloroform and worked up in the usual way.[26] The crude gum, wt. 47 g, was chromatographed over 500 g of silicic acid (Mallinckrodt 100 mesh), 300 ml fractions being collected. Elution with benzene to benzene-$CHCl_3$ (1:3; fractions 1-7) gave 2.6 g of gummy mixture. Elution with benzene-$CHCl_3$ (1:3) to $CHCl_3$ (fractions 8-23) gave 10.5 g of crude hymenogrannin (1g) which was recrystallized from acetone-ether, mp 149-150°, $[\alpha]_D$ +80.7° (c 1.48). Hymenogrannin formed solvates with ethyl acetate and acetone which initially complicated interpretation of the nmr spectrum. However, repeated recrystallization from methanol-water eliminated the problem and furnished crystals which melted at 153-154°, uv λ_{max} 215 nm (ε 14000), ir bands at 3450 (hydroxyl), 1760 (γ-lactone), 1734, 1712 (ester) and 1660 cm^{-1} (double bond), nmr signals (90 MHz, $CDCl_3$) at 4.83-5.08 ppm (2 overlapping protons, H-2 and H-3), 3.62d (5.0, H-4), 3.19m (H-7), 4.77m (H-8), 5.62d (2.0) and 6.27d (2.5, H-13), 1.08d (6.5, H-14), 0.97 (H-15), 2.08 and 2.03 (acetates), CD curve (0.7 mg/ml), $[\theta]_{300}$ 0; $[\theta]_{275}$ -1950; $[\theta]_{265}$ -2790; $[\theta]_{255}$ -3450 (min); $[\theta]_{240}$ -1730; $[\theta]_{236}$ 0; $[\theta]_{230}$ +920 (last reading).

Anal. Calcd for $C_{19}H_{26}O_7$: C, 62.98; H, 7.16; O, 30.57; MW 366. Found: C, 62.88; H, 7.14; O, 30.71; MW (chemical ionization), 366.

The high resolution mass spectrum of hymenogrannin lacked a peak corresponding to the molecular ion, but exhibited significant peaks at 324.1587 (2.5%, M-C_2H_6O), 306.1436 (base peak, M-$C_2H_4O_2$), 264.1364 (75%, M-C_4H_8O-C_2H_2O) and 246.1246 (65.2%, M-$2C_2H_4O_2$).

Elution with $CHCl_3$-MeOH (99:1, fractions 24-27) gave a gummy mixture. Elution with $CHCl_3$-MeOH (97:3, fractions 28-29) gave 1.67 g of crude florigrandin which after recrystallization from acetone-ether had mp 173-175°, $[\alpha]_D$ 187° (c 1.44), ir bands at 3380, 3295 (hydroxyls), 1768 (γ-lactone) and 1734 cm^{-1} (double intensity, ester and cyclopentanone, uv λ_{max} 262 nm (120), nmr signals (90 MHz, $CDCl_3$) at 5.11td ($\delta^{27},\delta^{27},\gamma^{27}$, H-2), 3.23dc ($1\gamma^{27},\delta^{27}$,H-3b), 2.01dd ($1\gamma^{27},\gamma^{27}$,H-3b), 4.74m (H-8), 3.75br (2α, H-13, split into AB quartet centered at 4.26 ppm in pyridine-d_5), 1.16d (7, H-14), 1.06 (H-15), 1.19d (7) and 0.91t (7 methyls of ester side chain).

Anal. Calcd for $C_{20}H_{30}O_7$: C, 62.81; H, 7.91; O, 29.28; MW, 382. Found: C, 63.64; H, 7.67; O, 29.09; MW (mass spectrometry), 382.

Other significant peaks in the mass spectrum of florigrandin were 364 (M-H_2O), 352 (M-CH_2O), 325 (M-C_4H_9), 280 (M-$C_6H_{10}O_2$), 266 (M-$C_6H_{10}O_2$-CH_2), 262 (M-H_2O-$C_5H_{10}O_2$).

Fraction 30 ($CHCl_3$-MeOH, 97:3) gave a gummy mixture. Further elution with the same solvent (fractions 31 and 32) gave 8.2 g of crude hymenoflorin which was recrystallized from acetone and then had mp 197-199°, $[\alpha]_D$ -64.3° (c 0.92), ir bands at 3540-3700 (broad hydroxyl), 1772 (γ-lactone), 1700 and 1674 cm^{-1} (cyclopentenone), uv λ_{max} 217.5 nm (ε 9050), CD curve (0.49 mg/ml), $[\theta]_{380}$ 0; $[\theta]_{360}$ -2850; $[\theta]_{325}$ -5420 (min); $[\theta]_{300}$ -2660; $[\theta]_{275}$ -670 (max); $[\theta]_{250}$ -2090 (last reading), nmr signals (90 MHz, DMSO-d_6) at 7.76dd (6.0, 1.5, H-2), 6.06dd (6.0, 2.8, H-3), 4.78- (H-8), 3.5br (2p, H-13), 1.23d (6.5, H-14), 1.08 (H-15), 5.15- (6.0, -CH_2OH, disappears on D_2O exchange), 5.07 (3° OH, disappears on D_2O exchange).

Anal. Calcd for $C_{15}H_{20}O_5$: C, 64.27; H, 7.19; O, 28.54; MW, 280 Found: C, 66.22; H, 6.90; O, 28.29; MW, 280.

Other significant peaks in the mass spectrum were 266 (M-CH_2), 250 (M-CH_2O), 249 (M-CH_2OH), 235 (M-CH_3-CH_2O) and 232 (M-CH_2O-H_2O).

Further elution with $CHCl_3$-MeOH (93:7 and 19:1, fractions 33-36) gave a gummy mixture. MeOH-$CHCl_3$ (1:9, fractions 37-40) gave 2.6 g of crude paucin which was recrystallized from acetone, mp 177-179°, identical in all respects with material previously isolated from hymenoxys odorata DC.[3]

B) Repetition of the extraction with 9 kg of H. grandiflora collected by Professor F. R. Stermitz on July 6, 1972 in Rocky Mountain National Park 1/2 mile off Trail Ridge Road at an altitude of 11000 ft with permission of the National Park Service (FRS-42 on deposit in herbaria of Colorado State University and Florida State University) gave 200 g of crude gum which partially crystallized on standing. Chromatography over 3.4 kg of silicic acid gave 80 g of hymenogrannin, 3.2 g of florigrandin, 6.2 g of hymenoflorin and 3.2 g of paucin.

Hydrolysis of Hymenogrannin. -- A solution of 0.8 g of 1g in 15 ml of methanol and 5 ml of water containing 0.385 g of Na_2CO_3 was allowed to stand at room temperature for 20 min, diluted with water, acidified with dil. HCl and extracted with ethyl acetate. The washed and dried extract was concentrated and the residue was chromatographed over silica gel. Elution with $CHCl_3$-MeOH (9:1) gave 1c as a colorless gum which could not be crystallized, mol. wt. (mass spectrum) 282, calcd for $C_{15}H_{22}O_5$, 282; nmr peaks (60 MHz, acetone-d_6), complex system of 3 protons in range 4.36-3.40 ppm (H-2, H-3 and H-4), 3.07m (H-7), 4.72m (H-8), 5.52d (2) and 5.97 (2.5, H-13), 1.18d (6.0, H-14), 0.83 (H-15).

Acetylation of 0.21 g of 1c with pyridine-acetic anhydride at room temperature and work-up in the usual way gave a gum which was chromatographed over 5 g of silica gel. Elution with benzene-chloroform (1:1) gave a triacetate 1a, wt. 0.185 g, which could not be induced to crystallize and was identical with material prepared by direct acetylation of 1a, $[\alpha]_D$ +76.9 (c 1.30), ir bands ($CHCl_3$) at 1768, 1750 (very strong) and 1668 cm^{-1} (double bond), nmr signals (60 MHz, $CDCl_3$) complex system of 3 protons in range 4.90-5.30 ppm (H-2, H-3 and H-4), 3.17- (H-7), 4.73m (H-8), 5.50d (2.0) and 6.17d (2.5, H-13), 1.07d (6.5, H-14), 1.00 (H-15), 1.97, 2.03, 2.08 (acetates).

Anal. Calcd for $C_{21}H_{28}O_8$: C, 61.76; H, 6.91; O, 31.34. Found: C, 61.62; H, 6.98; O, 31.50.

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Treatment of 1c with acetone-toluenesulfonic acid gave a quantitative yield of the acetonide 2 (vide infra).

Preparation of 2. -- A solution of 0.316 g of 1a in 4 ml of acetone-water (1:1) and 10 ml of conc. HCl was allowed to stand at room temperature overnight, diluted with water and extracted with ethyl acetate. The washed and dried solvent was evaporated; the residue (2) was recrystallized from ethyl acetate, mp 168.5-170°, $[\alpha]$ +80.9° (c 1.05), ir bands ($CHCl_3$) at 3640, 3585 (OH), 1764 and 1658 cm^{-1} (conjugated γ-lactone), nmr signals (90 MHz, $CDCl_3$) 4.26dd (6.5, 8, H-2), 4.41dd (8, 5, H-3), 3.76d (5, H-4), 3.21m (H-7), 4.77m (H-8), 6.26d (2.4, H-13a), 5.59d (2.2, H-13b), 1.13d (6.5, H-14), 0.92 (H-15), 1.49 and 1.29 (acetonide methyls).

Anal. Calcd for $C_{18}H_{26}O_5$: C, 67.06; H, 8.13; O, 24.81. Found: C, 66.78; H, 8.25; O, 25.16.

Oxidation of 0.096 g of 2 in 1 ml of pyridine by treatment with 0.066 g of CrO_3 in 1 ml of pyridine overnight followed by dilution with water, extraction with ethyl acetate and concentration of the washed and dried extract in vacuo gave 0.087 g of 3 which was recrystallized from ethyl acetate, mp 176.5-180°, $[\alpha]_D$ +58.3° (c 1.235), ir bands ($CHCl_3$) at 1760 (strong, lactone and cyclopentanone) and 1680 cm^{-1}, nmr signals (90 MHz, $CDCl_3$) at 4.58t (8.0, H-2), 4.89d (8.0, H-3), 3.10m (H-7), 4.80m (H-8), 5.71d (2.0) and 6.27d (2.5, H-13), 1.25d (6.5, H-14), 1.09 (H-15), 1.36 and 1.48 (methyls of acetonide).

Anal. Calcd for $C_{18}H_{24}O_5$: C, 67.48; H, 7.55; O, 24.97. Found: C, 66.90, H, 7.27; O, 24.81.

Oxidation of Hymenogrannin. -- A solution of 0.25 g of 1e in 2 ml of pyridine was allowed to stand at room temperature with 0.23 g of CrO_3 in 1.5 ml of pyridine for 2 days. The work-up described in the previous paragraph gave a gum which was chromatographed over 5 g of silica gel. Elution with benzene-chloroform (4:1) gave crystalline 4 which was recrystallized from ethyl acetate, mp 144-146°, $[\alpha]_D$ +19.2° (c 0.626), ir 1760 and 1655 (unsaturated lactone), 1730 (acetate), 1720 and 1610 cm^{-1} (cyclopentanone), uv λ_{max} 214 and 240 nm (ε 11000 and 3400), nmr signals (60 MHz) at 7.00d (1.5, H-2) 3.32m (H-7), 4.79 m (H-8), 5.55d (2.0) and 6.16d (2.5, H-13), 1.23d (6.0, H-14), 1.23 (H-16), 2.21 ppm (acetate).

Anal. Calcd for $C_{17}H_{20}O_5$: C, 67.09; H, 6.62; O, 26.28; MW, 304.1310 Found: C, 67.12; H, 6.64; O, 26.26; MW, 304.1332.

Other significant peaks in the mass spectrum were at 262.1210 (base peak, M-C_2H_6O), 244.1095 (11.5%, M-$C_2H_4O_2$) and 234.1245 (24.5%, M-C_2H_6O-CO).

Diacetylflorigrandin (5b). -- Acetylation of 0.1 g of 5a in 1 ml of pyridine with 0.6 ml of acetic anhydride at room temperature overnight and work-up in the usual way afforded 0.12 g of crude 5b which was recrystallized from ethyl acetate, mp 161°, $[\alpha]_D$ +75° (c 1.00), nmr signal (90 MHz, $CDCl_3$), at 5.12td (8, 8, 7, H-2), 3.26dd (19.5, 8, H-3a), and 2.08m (H-3b), 2.6m (H-7), 4.74m (H-8), 4.34 (center of AB quartet, J = 11, H-13), 1.17c (6.5, H-14), 1.01 (H-15), 1.20d and 0.92t (methyls of side chain).

Anal. Calcd for $C_{24}H_{34}O_8$: C, 61.79; H, 7.35; O, 30.86; MW, 466 Found: C, 61.62; H, 7.44; O, 30.95; MW, 466.

Other significant peaks in the mass spectrum were 451 (M-CH_3), 437 (M-C_2H_3), 424 (M-C_2H_6O), 409 (M-C_4H_9), 406 (M-$C_2H_4O_2$), 394 (M-C_2H_8O-CH_2O), 382 (M-$2C_2H_4O$), 364 (M-$C_6H_{10}O_2$), 346 (M-$2C_2H_4O_2$), 322 (M-$C_6H_{10}O_2$-C_2H_2O), 304 (M-$C_6H_{10}O_2$-$C_2H_4O_2$), 280 (M-$C_6H_{10}O_2$-$2C_2H_2O$), 262 (M-$C_6H_{10}O_2$-$2C_2H_4O$), 244 (M-$C_6H_{10}O_2$-$2C_2H_4O_2$).

Conversion of Florigrandin to Diacetylhymenoflorin. -- A solution of 0.045 g of 5a in 2 ml of MeOH and 0.6 ml of conc HCl was allowed to stand at room temperature overnight, diluted with water and extracted with ethyl acetate. The washed and dried extract was evaporated in vacuo and the residue was acetylated with acetic anhydride-pyridine in the usual manner. Purification by preparative tlc yielded a gummy product which was identical in every respect with authentic 6b.

Diacetylhymenoflorin (6b). -- Acetylation of 0.1 g of 6a with 0.4 ml of acetic anhydride and 1.5 ml of pyridine in the usual way followed by preparative tlc of the crude product gave a gum which could not be induced to crystallize, nmr signals (270 MHz) in Table III, ir bands at 1760 (γ-lactone), 1735 (ester), 1680 and 1675 cm^{-1} (cyclopentenone).

Anal. Calcd for $C_{19}H_{24}O_7$: C, 62.63; H, 6.64; O, 30.73 Found: C, 62.08; H, 6.68; O, 30.73.

Dibenzoylhymenoflorin (6c). -- Benzoylation of 0.1 g of 6a with 0.16 g of benzoyl chloride in 2 ml of pyridine at 0° overnight followed by the usual work-up gave a gum which was purified by preparative tlc and had nmr signals (90 MHz, $CDCl_3$) at 7.5m (H-2, partially superimposed on 7C aromatic protons), 6.03dd (6.0, 2.5, H-3), 2.52dd (15, 4.5, H-6), 1.56t (15, 13.5, H-6), 3.06m (H-7), 4.88m (H-8 partially superimposed on H-13), 4.69 (center of AB quartet, J = 11, H-13), 1.17d (H-14), 1.04 ppm (H-15), CD curve (0.28 mg/ml, MeOH), $[\theta]_{375}$ 0; $[\theta]_{350}$ -2190; $[\theta]_{325}$ -4720 (min); $[\theta]_{300}$ -2190; $[\theta]_{280}$ 0; $[\theta]_{250}$ -1960 (last reading).

Anal. Calcd for $C_{29}H_{28}O_7$: C, 71.30; H, 5.78; O, 22.92; MW, 488.1 Found: C, 72.05; H, 5.45; O, 22.71; MW, 488.1835.

Other significant peaks in the mass spectrum were at 366.1031 (M-$C_7H_6O_2$) and 244.1046 (M-$2C_7H_6O_2$).

Preparation of 7a. -- A mixture of 0.075 g of 6a and 0.15 g of anhydrous zinc chloride in 4 ml of acetaldehyde was left overnight at room temperature, concentrated in vacuo and extracted with ethyl acetate. The washed and dried extracts were evaporated and the solid residue of 7a, wt. 0.07 g, was recrystallized from ethyl acetate, mp 190-193°, $[\alpha]_D$ -87.8° (c 0.64), ir bands at 1783 (γ-lactone), 1705 and 1584 cm^{-1} (cyclopentenone), nmr signals (60 MHz) at 7.56dc (6.0, 1.5, H-2), 6.18dd (6.0, 2.0, H-3), 4.8-m (H-8), 4.08 (2p, center of AB

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quartet, H-13), 1.27d (6.0, H-14), 1.27 (H-15), 5.20q (6.0, methinyl of ethylidene), 1.49c (5.0, methyl of ethylidene).

Anal. Calcd for $C_{17}H_{22}O_5$: C, 66.65; H, 7.24; O, 26.11 Found: C, 66.25; H, 7.27; C, 26.30.

Preparation of 7b. -- Thionyl chloride (0.5 ml) was added dropwise with stirring to a solution of 0.11 g of 6a in 1.5 ml of pyridine at 3°. After an additional ten minutes, the mixture was poured into ice water and extracted with ethyl acetate. The washed and dried extracts were evaporated; the residual gum was chromatographed over 5 g of silica gel. Benzene-$CHCl_3$ (1:1) eluted solid 7b which was recrystallized from ether-ethyl acetate, mp 146-148°, $[\alpha]_D$ -55.6° (c 0.90), ir bands at 1780, 1710 and 1594 cm^{-1}, nmr signals (60 MHz, $CDCl_3$) at 7.40dd (6.0, 2.0, H-2), 6.02dd (6.0, 2.5, H-3), 4.77m (H-8), 4.70 (2p, center of AB quartet of H-13), 1.27d (5.0, H-14), 1.22 (H-15).

Anal. Calcd for $C_{19}H_{18}O_6S_2$: C, 55.20; H, 5.56; O, 29.41 Found: C, 54.80; H, 5.60; O, 29.47.

Dihydrohymenoflorin (8). -- A solution of 0.32 g of 6a in 20 ml of EtOAc was hydrogenated in the presence of 0.12 g of 10% Pd-C at room temperature and atmospheric pressure until hydrogen uptake ceased. Filtration followed by evaporation at reduced pressure gave solid 8, wt. 0.215 g, which was recrystallized from acetone, mp 192-193°, $[\alpha]_D$ -72° (c 1.36), ir bands at 3450, 3365 (hydroxyls), 1770 (γ-lactone) and 1735 cm^{-1} (cyclopentanone), nmr signals (60 MHz in DMSO-d_6) at 4.75m (H-8), 3.50 (2p, AB quartet of H-13 after addition of D_2O), 1.05d (6.0, H-14), 0.88 (H-15), 5.33t (6.0, primary -OH), 5.73 (tertiary OH), CD curve (0.3 mg/ml), $[\theta]_{350}$ 0; $[\theta]_{320}$ +96; $[\theta]_{295}$ -586 (max); $[\theta]_{275}$ +237, $[\theta]_{250}$ +13 (min); $[\theta]_{227}$ 42 (max), $[\theta]_{218}$ 0 (last reading).

Anal. Calcd for $C_{15}H_{22}O_5$: C, 63.81; H, 7.85; O, 28.33; MW, 282. Found: C, 64.06; H, 7.51; O, 28.30; MW, 282.

Other significant peaks in the mass spectrum were at 1252 (base peak, M-CH_2O) and 233 (M-CH_3-CH_2O).

The gummy acetonide could not be induced to crystallize.

Periodate Oxidations. -- A) A solution of 0.12 g of 6a in 2 ml of MeOH and 0.1 g of sodium metaperiodate in 1 ml of methanol and 0.3 ml of water was allowed to stand overnight, diluted with water and extracted with ethyl acetate. The washed and dried extract was evaporated and the solid residue (9) was recrystallized from acetone, yield 0.105 g, mp 247-249°, $[\alpha]_D$ -110° (c 1.22), ir bands at 3230 (hydroxyl), 1760 (α,β-unsaturated lactone, 1686 and 1575 cm^{-1} (α,β-unsaturated cyclopentenone), uv λ_{max} 231 nm (ε 21300), nmr signals (60 MHz, DMSO-d_6) at 8.00dd (6.0, 2.0, H-2), 6.32dd (6.0, 2.5, H-3), 5.25t (7.5, 6.0, H-8), 1.32d (6.0, H-14), 1.00 (H-15), 9.88br (enolic-OH).

Anal. Calcd for $C_{14}H_{16}O_4$: C, 67.73; H, 6.50; O, 25.78 Found: C, 68.26; H, 5.29; O, 25.29.

B) Oxidation of 0.11 g of 8 with sodium metaperiodate in the same manner and recrystallization from acetone afforded the enol lactone 10, mp 217-219°, $[\alpha]_D$ +61° (c 1.09), ir bands at 3480, 3370 (hydroxyl), 1760 (unsaturated lactone) and 1734 cm^{-1} (cyclopentanone), uv λ_{max} 237 nm (ε 22400), nmr signals (90 MHz, DMSO-d_6) at 3.24d (15.2, H-6a), 4.94t (7, H-8) 1.23d (6.0, H-14), 0.98 (H-15).

Anal. Calcd for $C_{14}H_{18}O_4$: C, 67.18; H, 7.25; O, 25.57 Found: C, 67.54; H, 7.27; C, 24.96.

Table III
Nmr Spectrum of 6b[a]

H-1	2.22 m	$J_{1,10} \cong 10$[b]
H-2	7.51 dd	$J_{1,2} = 1.5$, $J_{2,3} = 6$
H-3	6.08 dd	$J_{1,3} = 2.5$
H-6α	2.39 dd	$J_{6\alpha,6\beta} = 15$, $J_{6\alpha,7} = 4.5$
H-6β	1.48 t	$J_{6\beta,7} = 13.5$
H-7	2.74 m	$J_{7,8} = 7$
H-8	4.77 septet	$J_{8,9\alpha} = 3.5$, $J_{8,9\beta} = 11$
H-9α	2.45 m	$J_{9\alpha,10} = 0.8$
H-9β	2.66 m[c]	
H-10	2.05 m	$J_{10,14} = 6.5$
H-13[d]	4.31 br[e]	
H-14[f]	1.26 d	
H-15[f]	1.14	
Acetates	2.19, 2.09	

[a] Run at 270 MHz in $CDCl_3$ with TMS as internal standard. Signals are given in parts per million, coupling constants in hertz. Multiplicities are indicated by the usual symbols. [b] Estimate from line width of H-1 when H-2 and H-3 were decoupled. [c] $J_{9\beta,10}$ and $J_{9\alpha,9\beta}$ could not be determined satisfactorily. [d] Two protons. [e] Center of AB quartet. [f] Three protons.

reduction to dihydrohymenoflorin. Because of the large value of $J_{1,10}$, the C-10 methyl group must be α as is the case in all other pseudoguaianolides from *Hymenoxys* and related species. Hence the supposition that the C-7 side chain is β oriented as in other substances of this type is logical.

Cis fusion of the lactone ring in hymenoflorin and florigrandin was deduced as follows. First, the observed coupling constants for the seven-membered rings of **2** and **6b** were astonishingly similar (see Tables I and III). Secondly, construction of Dreiding models of **6b** with cis- and trans-fused lactone rings revealed that the observed coupling constants are satisfied if ring B of **6b** is in the boat form of a cis-fused lactone, while several observed coupling constants are at variance with coupling constants predicted from the measured dihedral angles in the two chair forms of the cis-fused lactone and the somewhat flexible chair form and the boat form of a trans-fused lactone.

Double-resonance experiments on florigrandin established $J_{1,2}$, $J_{2,3a}$, and $J_{2,3b}$ as 8, 8, and 7 Hz, respectively, but the orientation of the C-2 ester side chain could not be deduced with certainty from this information. Conclusive evidence for the existence of a cis relationship between H-2 and the C-5 methyl group, *i.e.*, for the β orientation of H-2, was provided by the demonstration of a relatively strong NOE arising from the spatial proximity of these two groups. Irradiation at the frequency of the C-5 methyl group produced, for **5b**, a 19.6% enhancement in the integrated intensity of H-2, but no enhancement in the intensity of the H-8 signal. The absence of an NOE between H-8 and the C-5 methyl group can be taken as additional evidence for a cis-lactone ring fusion.

The remaining problem, that of determining the stereochemistry at C-11, could not be solved satisfactorily. An attempt to use the method of Nakanishi and coworkers[23] for determining the configuration of acyclic diols failed when it was found that the CD curve of **6a** after addition of $Pr(dpm)_3$ did not exhibit new maxima of opposite sign and equal magnitude near 310 and 280 nm. In an attempt to apply the dibenzoate chirality rule,[24] which depends on the signs of two Cotton effects near 225 nm produced by two interacting benzoate chromophores, the dibenzoate **6c** was prepared and exhibited the expected physical properties. However, the CD curve could not be measured satisfactorily below 250 nm, although the usual minimum near 325 nm was seen due to the n, π^* transition of the cyclopentenone chromophore.

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Registry No.—**1a**, 51292-55-8; **1b**, 51292-56-9; **1c**, 51292-57-0; **2**, 51292-58-1; **3**, 51292-59-2; **4**, 51292-60-5; **5a**, 51292-61-6; **5b**, 51292-62-7; **6a**, 51292-63-8; **6b**, 51364-37-5; **7a**, 51292-64-9; **7b**, 51292-65-0; **8**, 51292-66-1; **9**, 51292-67-2; **10**, 51292-68-3.

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(25) Melting points are uncorrected. Rotations were run in chloroform unless otherwise stated, uv spectra in 95% ethanol on a Cary Model 14 recording spectrophotometer, ir spectra as KBr pellets on a Perkin-Elmer Model 257 grating spectrometer, CD curves in absolute methanol on a Jasco Model ORD/UV recording spectrometer, mass spectra on a high-resolution MS-902 mass spectrometer at 70 meV, and nmr spectra on Varian A-60, Bruker HFX-90, and HFX-270 nmr spectrometers. Analyses were performed by Dr. F. Pascher, Bonn, Germany.
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(27) By spin decoupling.

Synthesis of 5α-Cholesta-7,24-dien-3β-ol and Cholesta-5,7,24-trien-3β-ol[1]

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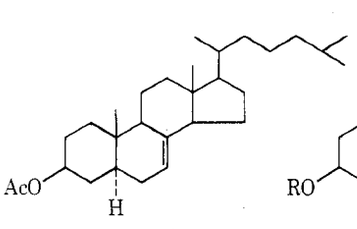
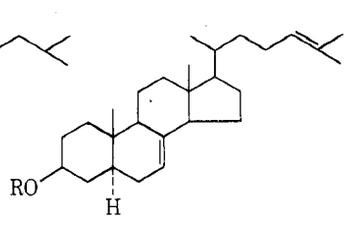
The title compounds were synthesized and were utilized for the identification of products of the *in vitro* incubation of mevalonic acid with yeast homogenates.

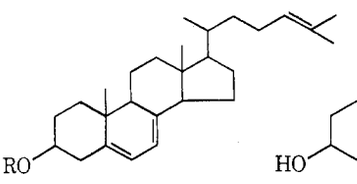
In the course of studies of the biosynthesis of sterols from (3*RS*,2*R*)-[2-^{14}C,2-^{3}H]mevalonic acid (MVA) and (3*RS*,2*S*)-[2-^{14}C,2-^{3}H]MVA in yeast homogenates, an unknown metabolite was obtained in a significant radioactive yield.[3] Frequently the metabolite contained *ca.* 20% of the total ^{14}C radioactivity of the nonsaponifiable residue. The acetate of the unknown on hydrogenation over nickel sponge in ethyl acetate[4] gave 5α-cholest-7-en-3β-ol acetate (**1**), thus revealing a C_{27} structure.[3] Analysis of the tritium content of the 7-en-3β-ols (**1**) derived from the *R* and the *S* metabolites indicated the incorporation in each case of four isotopic hydrogens. On theoretical grounds the presence of a tritium atom at C-26 of the metabolite and in **1** was assumed *a priori*. We have determined[3] the distribution of the isotopic hydrogens at C-1 and C-7 of **1** and have also deduced the distribution of ^{3}H at C-15. Based on our data it became clear that the metabolite retained both the 2-pro *R* and 2-pro *S* hydrogens of MVA at C-22. This establishes that the unknown does not have a C-22 double bond.[5] In view of the fact that the biosynthetic product had a C_{27} and *not* a C_{28} framework, it seemed reasonable to assume that it still retained the C-24 unsaturation required for the introduction of the 24-alkyl moiety.[6] The body of the available evidence suggested therefore either 5α-cholesta-7,24-dien-3β-ol (**2a**) and/or cholesta-5,7,24-trien-3β-ol (**3a**) as the likely structure for the metabolite.



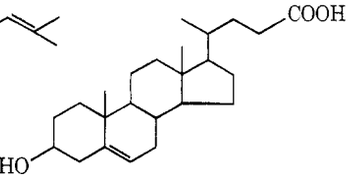
1



2a, R = H
b, R = CH_3CO



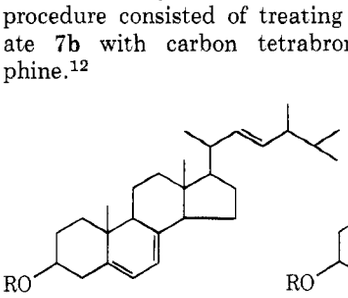
3a, R = H
b, R = CH_3CO



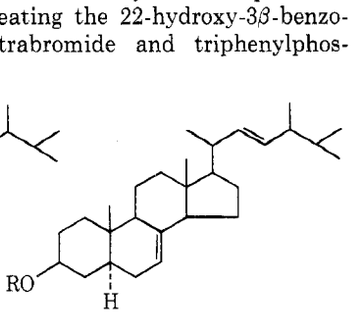
4

Cholesta-5,7,24-trien-3β-ol (**3a**) was previously prepared by Scallen.[7] Selective hydrogenation of the 5(6) double bond of **3a** afforded[8] 5α-cholesta-7,24-dien-3β-ol (**2a**). Since we required somewhat larger amounts of the diene **2a** and the triene **3a**, we undertook the preparation of these compounds and concentrated first on the synthesis of 5α-cholesta-7,24-dien-3β-ol (**2a**). We projected several approaches (*e.g.*, using **4** as starting material); however, the availability of ergosterol (**5a**) influenced our decision on a route *via* **7a** which we planned to couple with $(CH_3)_2C{=}CHCH_2X$.

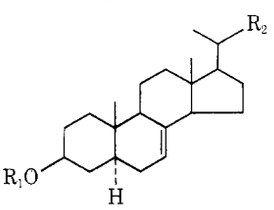
With this in mind, a benzene solution of ergosteryl benzoate (**5b**) was hydrogenated in the presence of tris(triphenylphosphine)rhodium chloride catalyst[9] to give 5α-ergosta-7,22-dien-3β-ol benzoate (**6**) in nearly quantitative yield. The diene **6** was dissolved in methylene chloride–pyridine[10] and ozonized at −78°. Following a reductive work-up, the aldehyde **7a** was isolated and subsequently reduced with sodium borohydride to the alcohol **7b**. The alcohol **7b** was converted to the bromide **7c** by two methods. The less convenient, two-step procedure involved the preparation first of the 22-tosyl ester 3β-benzoate **7d**. Displacement of the tosyl moiety was then carried out by warming a mixture of **7d**, lithium bromide, and dimethyl sulfoxide[11] to yield **7c** in *ca.* 70–75% yield. The preferred procedure consisted of treating the 22-hydroxy-3β-benzoate **7b** with carbon tetrabromide and triphenylphosphine.[12]



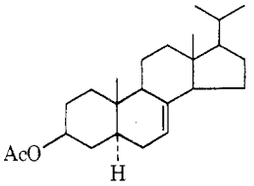
5a, R = H
b, R = C_6H_5CO
c, R = CH_3CO
d, R = p-$CH_3C_6H_4SO_2$



6, R = C_6H_5CO



7a, R_1 = C_6H_5CO; R_2 = CHO
b, R_1 = C_6H_5CO; R_2 = CH_2OH
c, R_1 = C_6H_5CO; R_2 = CH_2Br
d, R_1 = C_6H_5CO; R_2 = $CH_3C_6H_4SO_2$



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