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THE ISOLATION AND STRUCTURE DETERMINATION OF THE FERN GLYCOSIDE OSMUNDALIN AND THE SYNTHESIS OF ITS AGLYCONE OSMUNDALACTONE[1]‡

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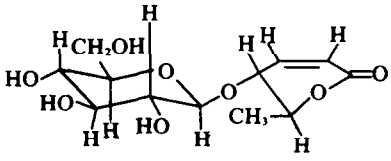
Abstract—From the Japanese foodstuff *Akaboshi zenmai*, consisting of dried leaves of *Osmunda japonica* Thunberg and from the Vermont royal fern *Osmunda regalis* var. *spectabilis* (Willd.) Gray a new hydroxypentenolide glucoside, osmundalin (1), was isolated in addition to β-sitosterol and methyl and ethyl palmitate. The structure and absolute stereochemistry of osmundalin were established by spectroscopic and degradation methods and its aglycone, osmundalactone, was synthesized.

An interest in a possible correlation of the unusually high incidence of gastric tumors in Japan[2] and unusual factors in the Japansse diet, prompted a study of chemical constituents of the fern *Osmunda japonica.* The young unopened fronds of this plant are eaten as a vegetable and the dried shoots are sold as the food *akaboshi zenmai.*

Thus, an abundant chemical constituent of this fern was isolated and characterized as a new Δ^2-pentenolide glycoside. While its carcinogenicity has not been established, it may be noted that other unsaturated lactones are carcinogenic (i.e., parasorbic acid, dehydroacetic acid, patulin, penicillic acid, and aflatoxins).[3]

Isolation and characterization of chemical constituents. Washing of dried *Osmunda japonica** with light petroleum, extraction with methanol and partitioning of the extract between ethyl acetate and water gave two fractions. Chromatography of the ethyl acetate soluble fraction on silica gel yielded methyl and ethyl palmitate and β-sitosterol. Adsorption of the aqueous fraction on activated charcoal and elution with hot methanol yielded the syrupy but practically pure new glycoside osmundalin (1) which was characterized as its crystalline tetraacetate $C_{20}H_{26}O_{12}$ (2). Retention of the original structure in the acetyl derivative was established by spectroscopic correlations (below) and by reconversion to osmundalin on mild hydrolysis.



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Osmundalin has an IR CO band at 1720 cm^{-1} which is also found in its acetyl derivative in addition to the acetate band at 1750 cm^{-1}.

The NMR spectrum shows single-proton absorptions at δ6·77 and 6·06 coupled to each other, $J_{2,3} = 10$ Hz, indicating a *cis*-olefinic function.[4] A multiplet at δ3·74 (1H) is assigned to the proton at C-5 of an acetylated pyranose moiety. This signal may be compared to the C-5 proton absorbance in the spectrum of methyl tetra-O-acetyl-β-D-glycopyranoside.[5] The acetoxy protons appear as four singlets at *ca* δ2·05 (12H).[6] A doublet absorption, $J_{5,6} = 6$ Hz, at δ1·48 (3 H) is due to a Me group coupled to a single proton. All other signals appear between δ5·35 and 4·20 (8H) and arise from protons attached to carbons bearing oxygen functions.

The mass spectrum (Fig 1) indicates an acetylated aldohexopyranoside. No molecular ion peak was observed; but M + 1, *m/e* 459, and M + 43, *m/e* 502, species could be discerned at high amplifier gain. These are produced by intermolecular capture of a proton or an acetylium ion, $[CH_3CO]^+$, respectively.[7] Peaks at *m/e* 331, 169, and 109 are prominent; they are characteristic of acetylated aldohexopyranoses that have substituents on the anomeric oxygen atom.[8] The most prominent peak at *m/e* 111 (34% of base peak at *m/e* 43) may be assigned to the unsaturated lactone fragment.

Extraction of some Osmunda species collected in Vermont showed the presence of large amounts of osmundalin in *O. regalis.*† but none could be found in *O. cinnamomia* or *O. claytoniana.*

*The plant material was identified by Dr. R. Tryon, curator of ferns, Gray Herbarium, Harvard University. We are greatly indebted to him for his friendly cooperation.

†The close relationship of O. japonica and O. regalis was noted on a herbarium sheet of the former, found at the Pringle Herbarium, University of Vermont, No. 1659, which was prepared from a specimen collected by Leopold A. Charette on Honshu Island in 1954, and confirmed by Dr. R. Tryon, Gray Herbarium, Harvard University.

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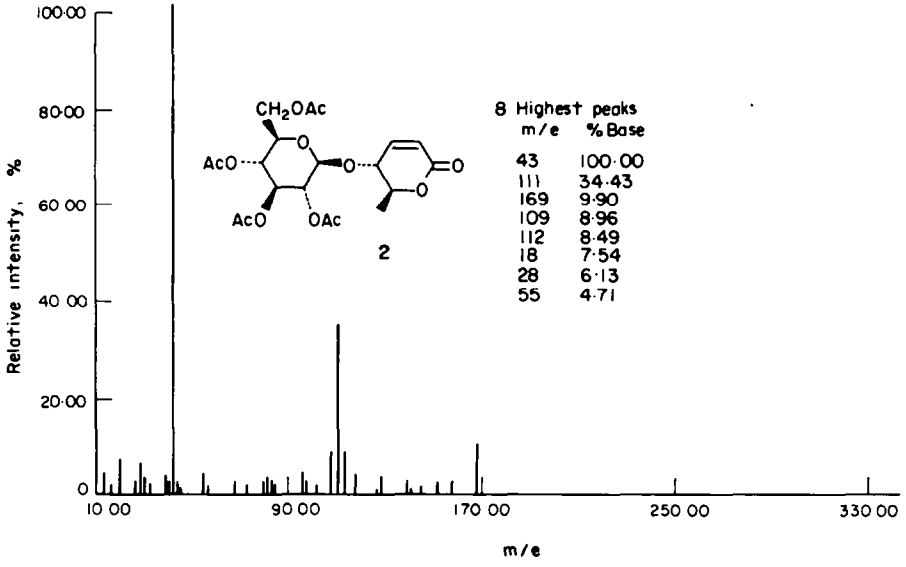


Fig 1. Mass spectrum of tetra-*O*-acetylosmundalin.

Hydrolytic cleavage products of osmundalin. Hydrolysis of tetra-O-acetylosmundalin in 8% sulfuric acid gave β-D-glucose and a low yield of a fragment, $C_6H_8O_3$ (3), which appears to have experienced molecular rearrangement. The 1720 cm^{-1} band is absent; but bands at 1783 and 1745 cm^{-1} are found in its IR spectrum. Such absorptions are indicative of α,β-unsaturated γ-lactones, i.e., Δ^2-butenolides.[9] Hydrogenation of the acid-hydrolysis product afforded a compound (4) showing CO absorption at 1768 cm^{-1}, characteristic of saturated γ-lactones.

An analysis of the 100 MHz NMR spectrum (Fig 2) could be matched with the structure 5-hydroxy-2-hexen-4-olide (3). The signal from H-3 appears at an unusually low-field position, δ7·60, for a vinylic proton, an effect of interaction with the CO group.[10] The magnitude of the vicinal coupling constant, $J_{2,3} = 5{\cdot}6$ Hz, is characteristic of *cis*-olefinic protons on a 5-membered ring.[4] Interestingly, the long-range allylic coupling constant, $J_{2,4} = 1{\cdot}8$ Hz, is greater in absolute value than is the vicinal coupling constant, $J_{3,4} = 1{\cdot}4$ Hz; this phenomenon has been observed in the case of the parent butenolide.[11] The OH proton signal, not shown in Fig 2, appears as a broad singlet at δ4·62.

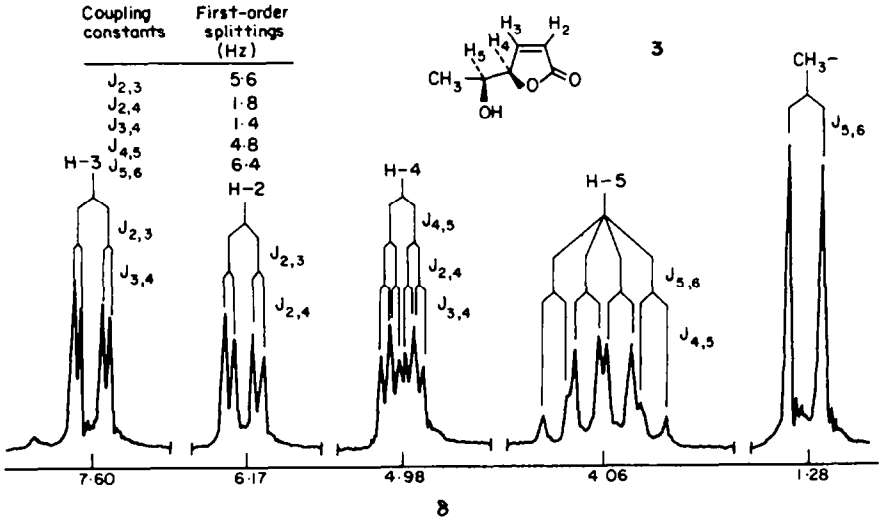


Fig 2. First-order analysis of the 100 MHz NMR spectrum of 5-hydroxy-2-hexen-4-olide (3).

Enzymatic hydrolysis of osmundalin with β-glucosidase from almond emulsin yielded osmundalactone **5**, the true aglycone of osmundalin, at a rate consistent with the cleavage of a β-D-glucopyranoside linkage. This crystalline, levorotatory $C_6H_8O_3$ fragment shows CO absorption at 1721 cm^{-1} in accord with the model α,β-unsaturated δ-lactone parasorbic acid (2-hexen-3-olide).

Comparison of the NMR spectrum of osmundalactone (Fig 3) with that of the acetylated glucoside confirmed that no rearrangement had accompanied cleavage. The vinylic proton signals appear at δ6·95 and 6·00 and show the same *cis*-olefinic coupling constant, $J_{2,3} = 10·0$ Hz. Additional coupling splits each of these signals forming a doublet of a doublet, $J_{2,4} = 1·8$ Hz and $J_{3,4} = 2·2$ Hz. A 3-proton signal, appearing as a doublet at δ1·52, $J_{5,6} = 6·2$ Hz, is assigned to a Me group. A singlet at δ4·00 arises from an OH proton, since it is absent from the spectrum of a D_2O solution. The values of the coupling constants are first-order splittings and must be considered tentative because the presence of a 2-proton, second-order complex in the region δ4·60–4·23 indicates strong coupling in the system, which appears to be of the type $ABMNX_3$. In order to extract the value of $J_{4,5}$, the critical parameter for assigning relative configuration, double resonance spin decoupling was applied. Irradiation of the Me doublet caused the low-field portion of the complex to reappear as a doublet, $J_{4,5} = 9·0$ Hz. This allows assignment of a 4,5-*trans* substituted Δ^2-pentenolide by comparison with analogous pentenolides to which *trans* (J = 9·0 Hz)[12] and *cis* (J = 2–3 Hz)[13–16] 4,5-substituted structures have been assigned. The secondary nature of the OH group was demonstrated by measuring the spectrum in DMSO-D_6 solution: the singlet at δ4·0 was deleted and a doublet at *ca* δ5·6 was introduced.[17]

Acetylation of the aglycone yielded O-acetylosmundalactone (**6**), $C_8H_{10}O_4$. Owing to the deshielding effect of the acetyl group on the geminal proton, the NMR spectrum of this derivative is amenable to first-order analysis (Fig 4). The acetyl signal, not shown in Fig 4, appears as a 3-proton singlet at δ2·19.

The absolute configuration of osmundalactone, shown in structure **3**, could be derived from its positive Cotton effect curve (Δξ max +3·86 at 263 nm)*.[18] which could be compared with that of (+) parasorbic acid (Δξ +2·25 at 262 nm)[19] since the latter compound had been correlated with 4,6-dideoxy-L-ribohexenolactone.[20]

Interestingly, the mass spectra of osmundalactone **5** (Fig 5) and its rearrangement product, the butenolide **3** (Fig 6) are nearly superimposable. However, the latter shows a molecular ion peak at *m/e* 128, whereas the former shows only an M + 1 peak. The base peak at *m/e* 84 (M − 44) was initially thought to arise by loss of CO_2 from the molecular ion, an important fragmentation process for saturated γ- and δ-lactones.[21] A high resolution measurement, however, revealed that this peak arises largely from an ion $[C_4H_4O_2]^+$ (*a*), produced by loss of acetaldehyde from the molecular ion.* Intense peaks at *m/e* 55 (*b*) and 56 (*c*) appear and are

*We are indebted to Dr. Thomas A. Narwid of Hoffmann-La Roche, Inc., Nutley, New Jersey, for obtaining the CD, ORD, and high resolution mass spectral measurements.

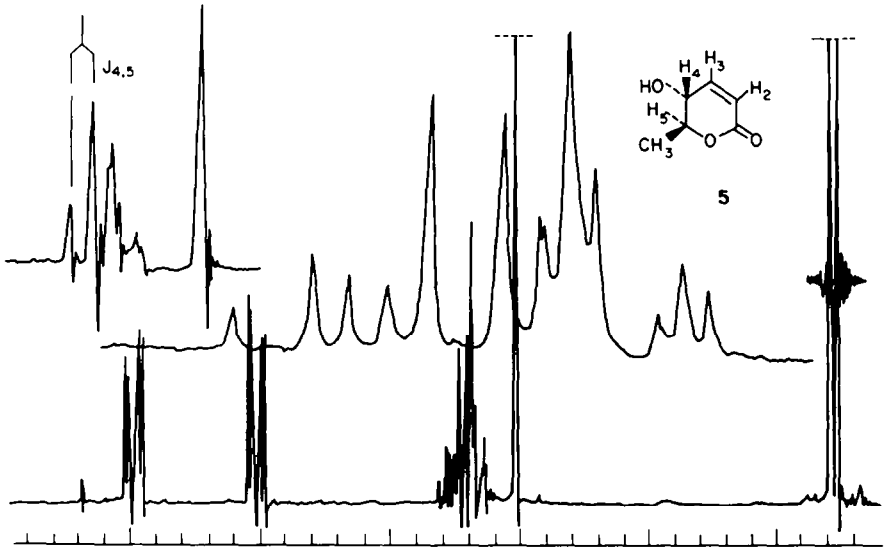


Fig 3. 100 MHz NMR spectrum of osmundalactone (**5**).

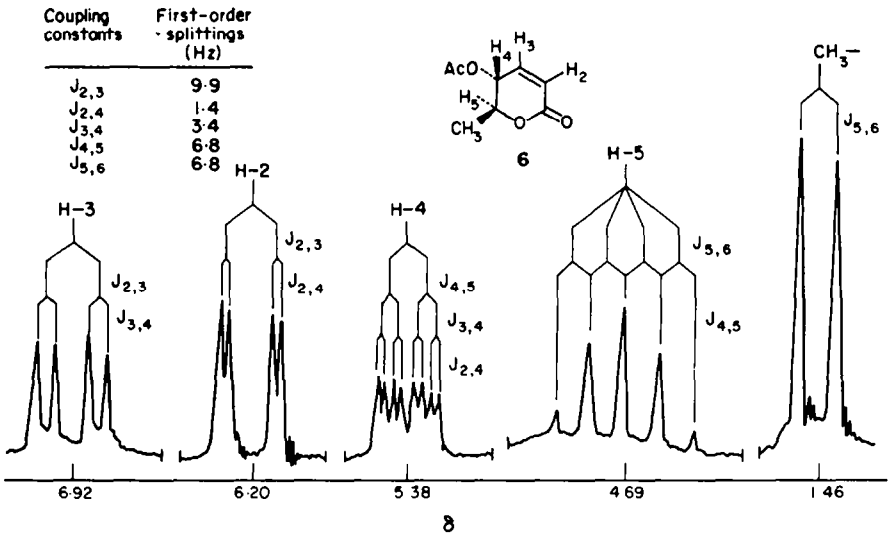
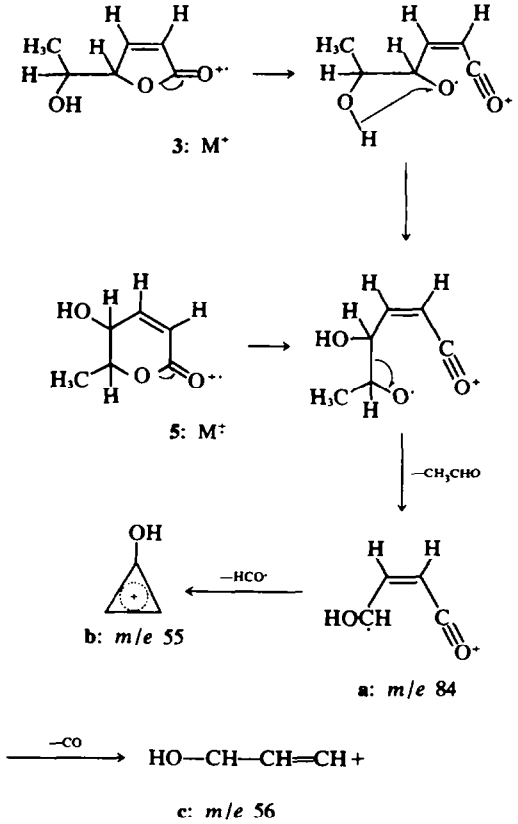


Fig 4. First-order analysis of the 100 MHz spectrum of *O*-acetylosmundalactone (6).

derived directly from ion *a*. Their origin is established by metastable peaks at *m/e* 36 [(55)²/84] and at *m/e* 37·4 [(56)²/84]. A tentative scheme that accounts for these data is shown below.



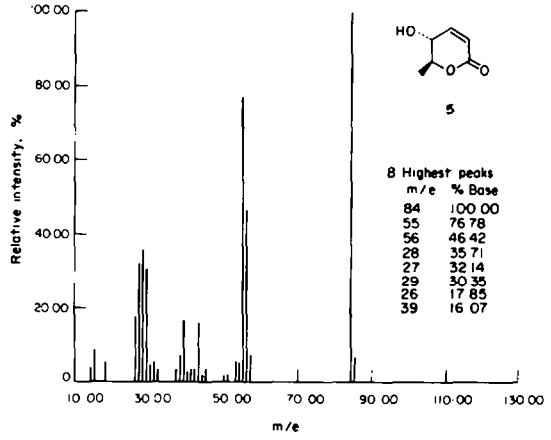


Fig 5. Mass spectrum of osmundalactone (5).

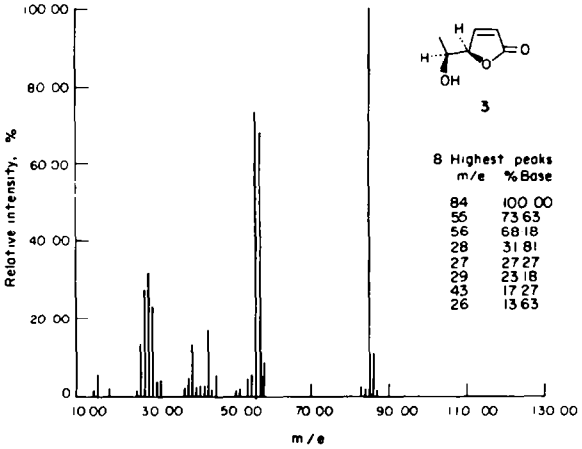


Fig 6. Mass spectrum of L-*erythro*-5-hydroxy-2-hexen-4-olide (3).

Chemical evidence in support of structure **5** for osmundalactone was obtained by the following observations: Catalytic hydrogenation gave a saturated δ-lactone **7** (IR 1727 cm^{-1})[22] that slowly rearranged on storage to the γ-lactone **4** (IR 1768 cm^{-1}), obtained on hydrogenation of the Δ^2-butenolide derived from acid hydrolysis of osmundalin. This ring contraction is consistent with a 4-hydroxyvalerolactone structure and the known preferred formation of γ-butyrolactones over δ-valerolactones under equilibrating conditions.[23] The two lactones **4** and **7** could be converted to a common dihydroxyhexanamide **8** by ammonolysis, thus establishing their common configuration.

Oxidation of dihydroosmundalactone with potassium permanganate yielded succinic acid as expected if both C-1 and C-4 carry oxygen functions. Reduction with LAH gave a dextrorotatory 1,4,5-hexanetriol, **9**. The position of the vicinal OH groups was established by a periodic acid cleavage that yielded acetaldehyde and 4-hydroxybutanal. The latter was identified by conversion to the known 2,4-dinitrophenylhydrazone and by mild oxidation with bromine to form γ-butyrolactone.[24]

The ring contraction observed on acidic hydrolysis of osmundalin could be duplicated on the isolated osmundalactone. In the presence of a catalytic amount of *p*-toluenesulfonic acid 75% of the osmundalactone rearranged in seven days to the hydroxyethylbutenolide **3** and all rearranged on longer treatment. In the absence of acid no γ-lactone formation was observed after 9 days. However, under basic conditions ring contraction was again found in accord with a previously described rearrangement of a hydroxypentenolide.[12]

Chemical confirmation of structure **5** for osmundalactone, with the shown relative and absolute stereochemistry, was obtained by its synthesis from L-rhamnose. Acetylation of this sugar to a mixture of the anomeric tetra-acetates **10** and reaction with 40% hydrogen bromide in acetic acid gave the rhamnopyranosyl bromide **11**, which with zinc and acetic acid yielded 3,4-di-O-acetyl-L-rhamnal (**12**)[25] in 87% yield overall. Heating the L-rhamnal in water gave 87% of 4-O-acetyl-2,3,6-trideoxy-L-erythro-hex-2-enopyranose **13** as an anomeric mixture. A previous report of this hydrolysis has appeared, but the product was not isolated.[26] Oxida-

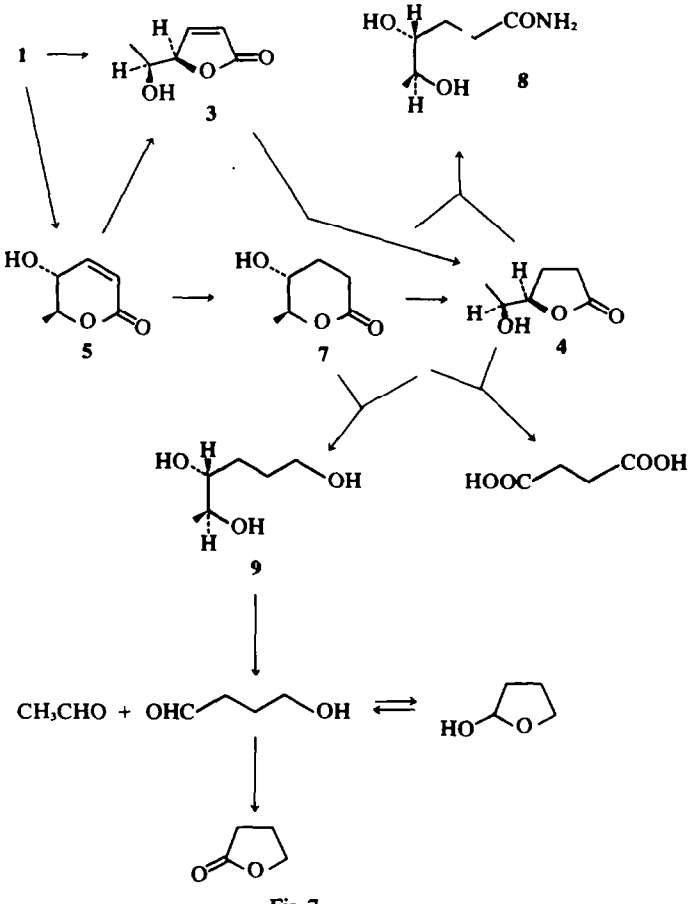


Fig 7.

tion of the allylic alcohol (13) was accomplished using active, neutral manganese dioxide prepared according to Henbest.[27] Yields were not dependable but varied from *ca* 5 to 45%. Such difficulty in controlling manganese dioxide oxidations has been noted by others.[28] Alternatively, oxidation with dipyridine-chromium trioxide gave dependable yields (*ca* 33%) of the desired Δ^2-pentenolide.[29]

The IR and NMR spectra of the Δ^2-pentenolide derived from L-rhamnose were superimposable with those of O-acetylosmundalactone. Furthermore, the specific rotations were identical within experimental error. Hence, osmundalactone has the L-*erythro* configuration.

The synthesis was completed by removing the 4-acetyl group with hot methanol containing a small amount of diisopropylethylamine. The crystalline product, obtained in 19% yield after chromatography, was identical with osmundalactone (5); m.p. and m.m.p. 81–82°.

Repetition of the sequence but starting with L-fucose led to the 4-epimer of the Δ^2-pentenolide already obtained. Thus, conversion of L-fucose to 3,4-di-O-acetyl-L-fucal[30] was accomplished in 53% yield overall. The hydrolysis to the hex-2-enopyranose mixture presented no difficulties and oxidation of the anomeric OH group, using the chromium trioxide reagent, gave the desired Δ^2-pentenolide, L-threo-4-acetoxy-2-hexen-5-olide. The NMR spectrum of this product confirmed its 4,5-*cis* substitution, $J_{4,5} = 2{\cdot}9$ Hz, and its mass spectrum was identical with that of the epimeric product obtained above.

EXPERIMENTAL

Extraction of Osmunda japonica. Dried leaves of *O. japonica* (Japanese, *akaboshi zenmai*), obtained commercially in Hawaii from Japanese food importers, were ground in a Wiley Mill to pass a 1 mm screen. The coarse powder (1400 g) was extracted in a Soxhlet apparatus with light petroleum (b.p. 35–60°) for 2 days. Evaporation of solvent gave a green, waxy substance (12 g). The plant

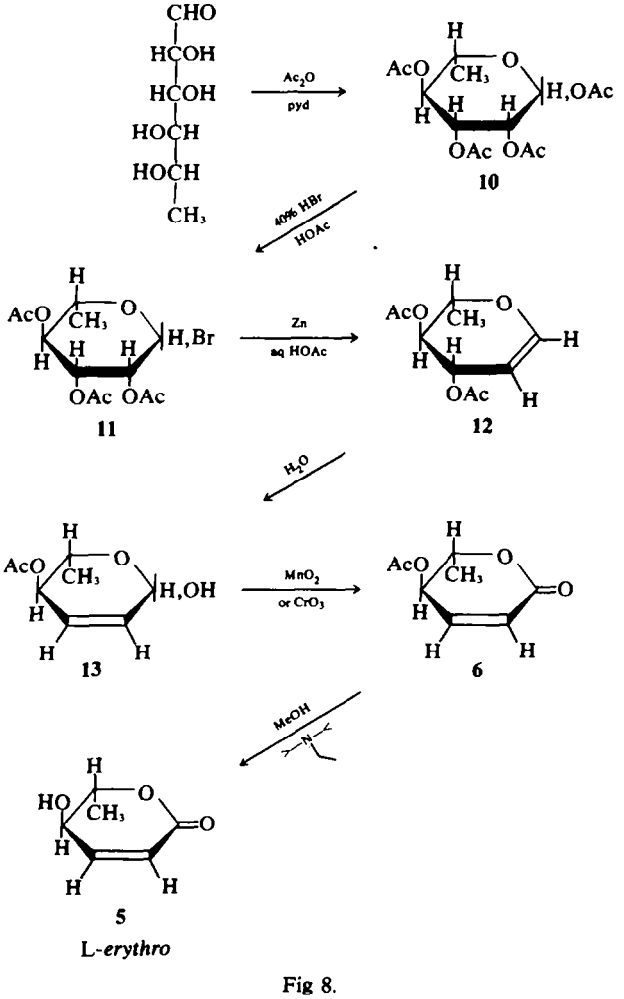


Fig 8.

material was extracted next with MeOH for 3 days; then the solvent was removed by continuous flash evaporation until frothing became unmanageable. To the green, viscous residue (*ca* 300 g) was added water (1000 ml) and EtOAc (1000 ml) and the equilibrated phases separated to yield a green EtOAc extract and a brown aqueous extract.

Palmitic acid esters. The EtOAc extract was washed with water and concentrated giving a dark, viscous residue. A portion (20·0 g) was chromatographed on a column (9 cm diam × 18 cm) of *Anasil* and eluted with chloroform. A yellow oil (0·186 g) obtained was distilled at 90–110° (10^{-3} mm). Preparative liquid chromatography of the yellow product with chloroform on a Corasil II column afforded a colorless oil, freezing point 8–11°. The IR and NMR spectra resembled those of fatty acid esters.[31] The mass spectrum indicated a mixture of methyl and ethyl palmitate: *m/e* (rel intensity) 284(4), 270(14), 88(31), 87(60), 74(100), 57(27), 43(53), 41(41), 29(22).

The oil was saponified by refluxing for 80 min in 2·5% alcoholic KOH. Recrystallization of the product from aqueous EtOH gave palmitic acid, m.p. and m.m.p. 60–61° (lit[32] m.p. 62·8°). The mass spectrum showed the molecular ion at *m/e* 256; ions of other homologs did not appear.

β-Sitosterol. Elution of the Anasil column was continued while small amounts of methanol were added gradually to the chloroform until the developing solvent contained 10% MeOH. A crystalline solid (0·225 g) was obtained, decolorized with *Norit-A*, and recrystallized twice from MeOH to give β-sitosterol, m.p. and m.m.p.[33] 136–137°. The IR spectrum was qualitatively identical to published spectra.[34,35]

Acetylation by Ac_2O pyridine gave a crystalline acetate, m.p. 123–124° (lit[33] 125–126°).

Osmundalin (1) *from* Osmunda japonica. The aqueous extract was treated with activated charcoal (125 g, *Nuchar-CN*, West Virginia Pulp and Paper Co.) and heated almost to boiling for 20 min. Then the slurry was filtered through diatomateous filter-aid (Celite). The filtrate was treated in an identical manner by two additional batchwise adsorptions. The combined charcoal (375 g) was washed with water (14 liters) and partially dried by suction. MeOH was added, and the slurry was heated with stirring for 20 min. The slurry was filtered, and the charcoal was again treated with hot MeOH. A total of five such desorptions yielded a yellow MeOH soln, which by flash evaporation gave osmundalin (22 g) as a brown, viscous syrup. Analysis by cellulose TLC using 1-butanol-acetic acid-water (4:1:1) revealed a large spot, R_f 0·55, which quenched the fluorescence of cellulose under UV light and reacted with alkaline $KMnO_4$ reagent and a small spot, R_f 0·38, which quenched fluorescence but did not react with alkaline $KMnO_4$. The osmundalin was sufficiently pure for all subsequent investigations. The brown color, however, could be removed by preparative paper chromatography using the same solvent system to yield a nearly colorless solid syrup; IR (thin film) 3344, 2899, 1718, 1631, 1391, 1287, 1247, 1198, 1156, 1075–1035, 892, 849, 815, and 746 cm^{-1}. The 100 MHz NMR spectrum (D_2O, HDO at 4·61) showed vinylic proton signals at δ7·06 and 6·08, and a 3-proton doublet at δ1·30. All attempts to crystallize osmundalin proved fruitless.

Tetra-O-acetylosmundalin. Osmundalin was acetylated by treatment with Ac_2O-pyridine reagent overnight at room temp. The mixture was poured into cold water and set aside for several h. The nearly white ppt was recrystallized from MeOH giving tetra-O-acetylosmundalin as colorless needles: m.p. 172·5–173·5°; IR (KBr) 2950, 1751, 1721, 1634, 1443, 1381, 1225 (broad), 1171, 1152, 1122, 1115, 1089, 1072, 1044, 976, 947, 907, 883, 839, 746, 723, and 690 cm^{-1}; NMR 100 MHz ($CDCl_3$) δ6·77 (d of d, 1H, J = 10, 2 Hz), 6·06 (d of d, 1H, J = 10, 1·5 Hz), 5·35–4·20 (complex, 8H), 3·74 (m, 1H), 2·11–2·03 (4 sharp s, 12H), and 1·48 ppm (d, 3H, J = 6 Hz); mass spectrum (Fig 1) *m/e* (rel intensity) 502(<1), 459(<1), 331(3), 169(10), 112(8), 111(34), 109(9), 55(5), 43(100), 28(6), 18(8). (Found: C, 52·16; H, 5·72. Calcd for $C_{20}H_{26}O_{12}$: C, 52·40; H, 5·72%).

Tetra-O-acetylosmundalin (20 mg) was dissolved in MeOH (10 ml), and 2% $KHCO_3$ (5 ml) was added. After being stirred at room temp for one week, the mixture was treated with activated charcoal and processed in the manner described above for isolating osmundalin. Analysis of the product by cellulose TLC revealed that impure osmundalin had been obtained.

Osmundalin (1) *from* Osmunda regalis. Pinnae (86·8 g, not dried) were removed from the axes of mature leaves of *O. regalis*, and were mascerated in a blender with MeOH. The pulp was extracted in a Soxhlet apparatus for 24 h; the MeOH was evaporated, and the residue was partitioned between water (100 ml) and EtOAc (150 ml). Drying ($MgSO_4$) and concentration of the EtOAc extract afforded a dark green residue (1·04 g). The aqueous extract was filtered to remove a gelatenous substance, evaporated under reduced pressure, and stored in a vacuum desiccator over P_2O_5 for 2 days to yield 6·34 g of extractive. This was dissolved in MeOH (5 ml); a portion was applied to one sheet of preparative chromatographic paper, and developed with 1-butanol-acetic acid-water (4:1:1). A band that showed quenching of fluorescence under UV light and reaction with alkaline $KMNO_4$ was cut out and eluted with hot MeOH. Solvent was removed giving a viscous syrup, which was acetylated with Ac_2O-pyridine. Recrystallization of the resulting derivative from MeOH yielded tetra-O-acetylosmundalin (0·105 g), m.p. and m.m.p. 171–172·5°.

Extraction of Osmunda cinnamomia. Dried, mature leaves of *O. cinnamomea* (483 g) were powdered and extracted by the same procedure as used for *O. japonica*. The aqueous extract was treated with activated charcoal in the same manner to yield a dark brown extractive (6·0 g). Analysis of this by cellulose TLC failed to reveal any osmundalin. Acetylation gave a yellow gum that could not be induced to crystallize.

Acidic hydrolysis of tetra-O-acetylosmundalin

(a) The acetylated glycoside (2·00 g) was refluxed for 20 min in 8% H_2SO_4 aq (50 ml). The soln was cooled and extracted with 5 portions of EtOAc. Concentration of the combined extracts and distillation at 130–140° (0·1 mm) yielded a yellow, fragrant oil (0·15 g, 27%). This was chromatographed on a column of silica gel and eluted with a solvent gradient starting with chloroform and ending with chloroform–ether (1:1). Fractions found to be homogeneous by TLC analysis were combined and distilled at 90–100° (0·05 mm) to give 3 as a colorless liquid: UV max (H_2O) 206 nm (ε 8400); IR ($CHCl_3$) 3497, 3344, 2924, 2857, 1783, 1745, 1603, 1451, 1383, 1339, 1304, 1170, 1100, 1059, 1047, 1019, 981, 952, 929, 916, 898, 873, 851, and 820 cm^{-1}; NMR 100 MHz ($CDCl_3$) δ7·60 (d of d, 2H, J = 5·6, 1·4 Hz), 6·17 (d of d, 1H, J = 5·6, 1·8 Hz), 4·98 (d of d of d, 1H, J = 7·8, 1·8, 1·4 Hz), 4·62 (broad s, 1H), 4·06 (d of quartet, 1H, J = 6·4, 4·8, Hz), and 1·28 ppm (d, 3H, J = 6·4 Hz); mass spectrum (Fig 5) *m/e* (rel intensity) 128(<1), 85(11), 84(100), 56(68), 55(74), 43(17), 39(13),

29(23), 28(32), 27(27), 26(14). (Found: C, 56·30; H, 6·50. Calcd for $C_6H_8O_3$: C, 56·25; H, 6·28%).

(b) Tetra-O-acetylosmundalin (0·500 g) was refluxed for 20 min in 8% H_2SO_4 aq (25 ml). The mixture was cooled, washed 3 times with EtOAc, and neutralized with NaOH aq. Water was evaporated under reduced pressure; the residue was treated with Ac_2O-pyridine overnight. Addition of cold water and refrigeration for several h yielded a white ppt (0·110 g), which was recrystallized from aqueous EtOH to give penta-O-acetyl-β-D-glucose, mp. and m.m.p. 131–132°. The IR spectrum was superimposable with that of the compound formed by acetylation of D-glucose with Ac_2O-sodium acetate.[36]

Enzymatic hydrolysis of osmundalin (1)

Rate study. To osmundalin (0·250 g) dissolved in the buffer a 0·1 M NaOAc buffer, pH 5·1 (20·0 ml), was added β-glucosidase enzyme (1·0 mg, Worthington E) dissolved in water (5·0 ml). At 35 ± 1°, aliquotes (0·10 ml) were removed at 5 min intervals and analyzed for glucose by Nelson's method to obtain colorimetric data.[37] The reaction reached equilibrium in 35 min.

Hydrolysis of osmundalin by β-glucosidase. Rate data

Aliquote	%T	A	Time (min)
1	100	0	0
2	61	0·22	5
3	46	0·34	10
4	30	0·52	15
5	21	0·68	20
6	18	0·74	25
7	19	0·72	30
8	16	0·80	35
9	16	0·80	40

Osmundalactone (5). To osmundalin (4·24 g) dissolved in 0·1 M NaOAc buffer (pH 5·1, 150 ml) was added a soln of β-glucosidase (20 mg) in buffer (50 ml). The mixture was left at room temp overnight and then extracted with five 50-ml portions of EtOAc. After an additional 24 h, the aqueous soln was again extracted and the combined organic solns dried ($MgSO_4$) and evaporated to yield a viscous, yellow oil that crystallized on seeding. Recrystallization from benzene afforded crude aglycone (0·58 g). Concentration of the mother liquor and crystallization gave additional product (0·51 g). The aglycone was purified by sublimation at 70–75° (10^{-3} mm) followed by recrystallization from benzene to give 5 as colorless, lustrous platelets: m.p. 82–82·5°; $[\alpha]^{22}$ D −70·6 (*c* 2·0, H_2O); UV max (H_2O) 200 nm (ε 10,500); IR ($CHCl_3$) 3584, 3413, 2985, 2907, 1721, 1634, 1456, 1391, 1289, 1170, 1130, 1104, 1062, 1025, 992, 965, 944, 889, 848, and 828 cm^{-1}; NMR 100 MHz ($CDCl_3$) δ6·95 (d of d, 1H, *J* = 2·2, 10·0 Hz), 6·00 (d of d, 1H, *J* = 1·8, 10·0 Hz), 4·60–4·23 (complex, 2H), 4·00 (s, 1H, shifts on dilution), and 1·52 ppm (d, 3H, *J* = 6·2 Hz); NMR 100 MHz (D_2O, HDO at δ4·61) δ6·99 (d of d, 1H), 6·00 (d of d, 1H), 4·53–4·17 (complex, 2H), and 1·33 ppm (d, 3H); mass spectrum (Fig 5) *m/e* (rel intensity) 129(< 1), 84(100), 56(46), 55(77), 43(16), 39(16), 29(30), 28(36), 27(32), 26(18), ORD (dioxane λnm ([φ])) start 700(0), 589(+12), inflection 292(+4125), peak 285(+4563), 268(0), trough 241(13625), 220(0), lost 210 (+7250); CD (dioxane) λnm ([θ]) start 301(0), inflection 272(+11500), max 263(+12750), 238(0), max 225(−7500), 212(0), lost 204(+13750). (Found: C, 56·09; H, 6·32; C-Me, 15·06, 14·43. Calcd for $C_6H_8O_3$: C, 56·25; H, 6·29; C-Me, 11·7%).

O-Acetylosmundalactone (6). Osmundalactone (0·500 g) was treated with Ac_2O (2 ml) and pyridine (2 ml) for 12 h. Cold water (50 ml) was added and allowed to react for 10 min. Extraction with four 20-ml portions of ether, evaporation of the ether, and distillation of the residue at 90–100° (0·1 mm) yielded a colorless oil (0·508 g, 82%), O-acetylosmundalactone (6): $[\alpha]^{24}$ D −172° (*c* 2·8 $CHCl_3$); IR ($CHCl_3$) 2924, 2865, 1730, 1631, 1447, 1389, 1377, 1350, 1285, 1160, 1127, 1115, 1089, 1057, 1035, 972, 958, 922, 888, 850, 842, 826, and 815 cm^{-1}; NMR 100 MHz ($CDCl_3$) δ6·92 (d of d, 1H, *J* = 9·9, 3·4 Hz), 6·20 (d of d, 1H, *J* = 9·9, 1·4 Hz), 5·38 (d of d of d, 1H, *J* = 6·8, 3·4, 1·4 Hz), 4·69 (quintet, 1H, *J* = 6·8 Hz), 2·19 (s, 3H), and 1·46 ppm (d, 3H, *J* = 6·8 Hz); mass spectrum *m/e* (rel intensity) 126(31), 84(78), 55(10), 43(100), 39(10), 27(9), 18(9), 15(8). (Found: C, 56·51; H, 6·16. Calcd for $C_8H_{10}O_4$: C, 56·47; H, 5·92%).

Conversion of osmundalactone (5) *to 5-hydroxy-2-hexen-4-olide* (3)

(a) To a deuteriochloroform soln of osmundalactone in an NMR sample tube was added *ca* 1 mg of *p*-toluenesulfonic acid monohydrate. After one week integration of the signals from vinyl protons revealed that the conversion to 5-hydroxy-2-hexen-4-olide had reached 75% completion. At 5 weeks no trace of osmundalactone remained.

(b) Osmundalactone (100 mg) was dissolved in 1 M NaOH and set aside for 30 min. After neutralization with dil HCl, the soln was extracted continuously for 4 h with ether–dichloromethane. Evaporation of the ethereal solution and distillation of the residue at 70–90° (0·05 mm) afforded 5-hydroxy-2-hexen-4-olide (22 mg). No trace of osmundalactone could be detected in the NMR spectrum.

Dihydroosmundalactone. Osmundalactone (0·735 g) was dissolved in dioxane (15 ml) and treated with H_2 in the presence of 10% Pd-C (60 mg). After 4 h an amount of H_2 (149 ml, 23°) had been absorbed corresponding to 1·1 moles per mole of unsaturated lactone. The catalyst was filtered off and washed with dioxane. Evaporation of the combined dioxane, and distillation of the residue at 105–110° (0·06 mm) gave dihydroosmundalactone (0·707 g, 95%) as a colorless liquid: $[\alpha]^{22}$ D −58·3° (*c* 2·0, H_2O); IR ($CHCl_3$) 3571, 3401, 2967, 2924, 1727, 1460, 1416, 1385, 1350, 1328, 1305, 1139, 1111, 1062, 1027, 984, 962, 942, and 918 cm^{-1}. The NMR spectrum ($CDCl_3$; 60 MHz) confirmed the absence of vinyl protons but revealed two methyl doublets, an intense one at δ1·35 ppm (*J* = 6·6 Hz) and a minor one at δ1·20 ppm (*J* = 6·6 Hz). The latter accounted for 27% of the total Me signal. (Found: C, 55·46; H, 7·80; C-Me, 15·86. Calcd for $C_6H_{10}O_3$: C, 55·37; H, 7·74; C-Me, 11·5%).

After storage at room temp for more than a year, the sample appeared to have rearranged completely to 4: IR ($CHCl_3$) 3484, 3356, 2915, 2874, 1767, 1475, 1420, 1385, 1368, 1323, 1152, 1076, 1032, 1013, 987, 916, 878, and 828 cm^{-1}; NMR 100 MHz ($CDCl_3$) δ4·42 (d of t, 1H, *J* = 3·5, 7·3 Hz), 4·10 (d of d, 1H, *J* = 6·6, 3·5 Hz), 2·67–2·03 (complex, 4H), and 1·21 ppm (d, 3H, *J* = 6·6 Hz).

Potassium permanganate oxidation of dihydroosmundalactone. Dihydroosmundalactone (0·382 g) was dissolved in 0·5 N H_2SO_4 (60 ml). At 15° finely powdered $KMnO_4$ (1·5 g) was added in small portions over 8 h with continuous stirring. SO_2 was then bubble through the mixture until the soln became clear. Excess SO_2 was

removed by a stream of N_2; and the soln was extracted continuously with ether–dichloromethane (4:1) for 34 h. Evaporation of the organic solvent and recrystallization of the residue from water yielded succinic acid (0·216 g, 62%), m.p. and m.m.p. 185–187°. The IR spectrum was superimposable with that of authentic material.

4,5-*Dihydroxyhexanamide* (8). An emulsion of dihydroosmundalactone (0·427 g) in benzene (4 ml) was treated for 0·5 h with dry ammonia. During 15 h a colorless oil separated and crystallized. Recrystallization from acetone gave 4,5-dihydroxyhexanamide (0·275 g, 57%) as lustrous platelets: m.p. 93–94°; $[\alpha]_D^{22}$ +27·2° (*c* 1·2, H_2O); IR (KBr) 3390, 3195, 2994, 2976, 1647, 1445, 1431, 1383, 1366, 1332, 1321, 1277, 1242, 1183, 1144, 1080, 1058, 992, 946, 906, and 884 cm^{-1}; NMR 60 MHz (DMSO-D_6) δ7·22 (s, 1H), 6·68 (s, 1H), 4·4 (2H), 3·6–3·0 (2H), 2·34–1·30 (complex, 4H), and 1·03 ppm (d, 3H, $J = 6$ Hz). (Found: C, 49·01; H, 9·13; N, 9·33. Calcd for $C_6H_{13}NO_3$: C, 48·97; H, 8·90; N, 9·52%).

1,4,5-*Hexanetriol* (9). A soln of dihydroosmundalactone (1·00 g) in dioxane (10 ml) was added dropwise to finely powdered LAH (2·00 g) suspended in dioxane (40 ml). The mixture was refluxed for 25 min when sudden H_2 evolution caused frothing; the heat source was removed for 5 min. This induction period followed by violent activity was observed with each repetition of this reaction. Reflux was continued for 3·5 days; then the mixture was cooled to 0°. Powdered $Na_2SO_4 \cdot 10H_2O$ (10 g) was added in small portions. After being stirred for 16 h, the mixture was filtered and the solid was washed with dioxane. Concentration and distillation of the residue at 100–120° (0·01 mm) gave 1,4,5-hexanetriol (0·88 g, 86%) as a viscous, slightly sweet liquid: $[\alpha]_D^{22}$ +20·6° (*c* 1·9, H_2O); IR (liquid film) 3279, 3899, 1639, 1449, 1416, 1374, 1175, 1136, 1055, 1013, 973, and 927 cm^{-1}.

The triol (80 mg) was heated with phenylisocyanate (1·0 ml) in an oil bath at 115° for 1 min. After 22 h at room temp, excess phenylisocyanate was removed by pumping at 0·3 mm leaving 315 mg of a white solid residue (293 mg theoretical). Recrystallization from acetone–hexane yielded the trisphenylurethane derivative, m.p. 182–182·5° after softening at 176°. (Found: C, 66·11; H, 5·95; N, 8·26. Calcd for $C_{27}H_{29}N_3O_6$: C, 65·98; H, 5·95; N, 8·55%).

Oxidative cleavage of 1,4,5-*hexanetriol* (9). A soln of the triol (0·463 g, 3·5 mmol) and periodic acid dihydrate (0·890 g, 3·9 mmol) in water (25 ml) was kept in the dark for 25 h. The soln was extracted continuously with ether–dichloromethane (4:1) for 7 h in an apparatus fitted with a cold-finger condenser (Dry Ice–isopropyl alcohol). The organic solvent was distilled through a Vigreux column into a cooled receiver. Semicarbazide hydrochloride (0·385 g, 3·5 mmol), pyridine (0·3 ml), and water (1 ml) were added and the mixture was shaken mechanically for 8 h. Evaporation of solvent gave a solid (70 mg, 20%) which crystallized on cooling from the remaining water. Recrystallization from water gave acetaldehyde semicarbazone, m.p. and m.m.p. 161–163°.

To the residue remaining after distillation of solvent was added a sol of 2,4-dinitrophenylhydrazine (0·685 g, 3·5 mmol), H_2SO_4 (2 ml), water (3 ml), and EtOH (10 ml). After 1 h the mixture was cooled and filtered. The solid was dissolved in EtOH (20 ml), and a red, insoluble impurity was removed by filtration. Concentration of the filtrate gave crystalline product (0·558 g, 60%); which was recrystallized from benzene and from EtOH to yield yellow γ-hydroxybutanal 2,4-dinitrophenylhydrazone, m.p. 120·5° (lit.[38] m.p. 120°). (Found: C, 44·76; H, 4·38; N, 21·16. Calcd for $C_{10}H_{12}N_2O_5$: C, 44·78; H, 4·51, N, 20·89%).

γ-*Butyrolactone*. The hydroxy aldehyde (100 mg) from periodate oxidation of the triol was dissolved in water (12 ml) and added to a cold mixture of Br_2 (1·2 ml), $BaCO_3$ (3·6 g), $BaBr_2 \cdot 2H_2O$ (3·6 g), and water (50 ml) saturated with CO_2. A slow stream of CO_2 saturated with Br_2 was bubbled through the mixture with magnetic stirring for 1 h. Then CO_2 was passed through for 2 h to remove most of the unreacted Br_2; the remainder was destroyed by treatment with 3% $Na_2S_2O_3$. The soln was filtered and extracted continuously for 14 h with ether–dichloromethane (4:1). Evaporation of the organic solvent and distillation of the residue at 86–90° (10 mm) afforded γ-butyrolactone, which gave IR and NMR spectra superimposable on those of authentic compound.

To the CCl_4 soln of the lactone used for NMR measurement was added phenylhydrazine (0·01 ml); the mixture was heated at reflux for 10 min, cooled, and light petroleum (1 ml) added to precipitate the product. Recrystallization from EtOH–$CHCl_3$ yielded 4-hydroxybutyric acid phenylhydrazide m.p. and m.m.p. 250–252° (sealed capillaries).

4-O*Acetyl*-2,3,6-*trideoxy*-αβ-L-*erythro*-*hex*-2-*enopyranose* (13). A soln of 3,4-di-O-acetyl-L-rhamnal[25] (20·4 g) in water (400 ml) was refluxed for 20 min. The soln was cooled quickly, treated with sat $KHCO_3$ aq (10 ml) and extracted continuously with ether for 12 h. The ethereal soln was dried ($MgSO_4$), concentrated, and the residue distilled at 95–104° (0·02 mm to give an anomeric mixture of 4-O-acetyl-2,3,6-trideoxy-L-*erythro*-hex-2-enopyranose (14·2 g, 87%) as a colorless viscous liquid. The 100 MHz NMR spectrum ($CDCl_3$) revealed multiplets of unit intensity at δ9·5, 6·9, 6·2, 5·4, and 4·1 ppm, as well as signals at 2·1 (3H) and 1·2 ppm (3H).

O-*Acetylosmundalactone* (6)

(a) To CrO_3-pyridine complex prepared from 19·0 g (240 mmol) of dry pyridine and 12·0 g (120 mmol) CrO_3 in 300 ml dichloromethane was added 3·44 g (20 mmol) L-erythrohexenopyranose in 20 ml dichloromethane. After 15 min the mixture was filtered, and the residue washed with dichloromethane (200 ml). The combined filtrates were concentrated to about 30 ml, and 250 ml of ether was added. Washing twice with 100-ml portions of 10% Na_2CO_3 aq and once with 100 ml sat NaCl aq gave a pale yellow soln. Drying, concentration, removal of the remaining pyridine, and distillation of the residue at 73° (10^{-2} mm) gave O-acetylosmundalactone (1·12 g, 33%) which appeared to contain a small amount of starting compound by TLC analysis. The product was purified by chromatography on a silica gel column and elution with $CHCl_3$–EtOAc (9:1). Fractions free of impurities were combined and redistilled.

(b) Active MnO_2[27] (111 g) was powdered and suspended in benzene (600 ml). Traces of water were removed by azeotropic distillation. To the cooled suspension 4-O-acetyl-αβ-L-*erythro*-hex-2-enopyranose (2·20 g) dissolved in benzene (25 ml) was added and the suspension was stirred vigorously at room temp for 3 h. The mixture was filtered, and the MnO_2 was washed with dry benzene. Evaporation of the combined benzene soln, and distillation of the residue at 73° (10^{-2} mm) gave O-acetylosmundalactone (0·99 g, 45%) as a pale yellow oil. TLC analysis showed the product to be free of starting compound. Yields from MnO_2 oxidation averaged about 30% for ten repetitions, and on only two occasions ex-

ceeded 40%. The NMR spectrum of the product was superimposable on that of acetylated osmundalactone. The specific rotation showed the two products to be identical in absolute configuration, $[\alpha]^{21}$ D −160° (*c* 3·1, $CHCl_3$). (Found: C, 56·37; H, 6·10. Calcd for $C_8H_{10}O_4$: C, 56·47; H, 5·92%).

Osmundalactone (5). O-Acetylosmundalactone (0·416 g) from L-rhamnose was refluxed for 24 h in dry MeOH (60 ml) containing 3 drops of diisopropylethylamine. Evaporation of the MeOH and distillation of the residue at 70–80° (0·02 mm) gave a product (0·245 g), which by TLC analysis appeared to be a mixture of the starting acetate and the desired alcohol. Chromatography on a column of *Anasil* and elution with chloroform yielded material that was free of the starting ester. Recrystallization from benzene (first crop, 40 mg; second crop, 20 mg; 19%) afforded osmundalactone, m.p. and m.m.p. 81–82°.

4-O-*Acetyl*-2,3,6-*trideoxy*-αβ-L-*threo*-*hex*-2-*enopyranose*. A soln of 3,4-di-O-acetyl-L-fucal[30] (6·88 g) was refluxed for 20 min in water (150 ml) and quickly cooled to 0°. Saturated $KHCO_3$ aq (50 ml) was added and the mixture was extracted continuously with ether for 5 h. The ether was dried ($MgSO_4$) and evaporated. Distillation of the residue at 84–102° (10^{-3} mm) afforded an anomeric mixture of 4-O-acetyl-2,3,6-trideoxy-L-*threo*-hex-2-enopyranose (4·83 g, 87%) as a pale yellow oil. The NMR spectrum was similar to that of the L-*erythro* mixture.

L-*threo*-4-*acetoxy*-2-*hexen*-5-*olide*. The L-*threo*-hexenopyranose acetate mixture (3·44 g) was oxidized by a soln of dipyridine–chromium(VI) oxide in dichloromethane by the same procedure that was used for the epimer. Distillation of product at 77° (0·02 mm) gave crude L-*threo*-lactone (1·64 g, 48%). As TLC analysis revealed the presence of starting compound the product was purified on a column of silica gel (30 g) and eluted with $CHCl_3$–EtOAc (9:1). Those fractions free of impurities were combined and distilled at 80° (0·02 mm) yielding L-*threo*-4-acetoxy-2-hexen-5-olide (0·814 g) as a pale yellow oil; NMR 100 MHz ($CDCl_3$) δ7·11 (d of d, 1H, *J* = 9·9, 5·8 Hz), 6·32 (d, 1H, *J* = 9·9 Hz), 5·32 (d of d, 1H, *J* = 5·8, 2·9 Hz), 4·81 (d of quartet, 1H, *J* = 6·8, 2·9 Hz), 2·19 (s, 3H), and 1·43 ppm (d, 3H, *J* = 6·8 Hz).

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