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δ-LACTONES OF POLYHYDROXY-C_{26} ACIDS IN *EUPATORIUM PILOSUM**

WERNER HERZ* and GANAPATHY RAMAKRISHNAN
Department of Chemistry, The Florida State University, Tallahassee, FL 32306, U.S.A.

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Key Word Index—*Eupatorium pilosum*; Compositae; δ-lactones of polyhydroxy-C_{26} acids; δ-lactones of *Z*-5(*R*),7,9,11-tetrahydroxy- and *Z*-5(*R*),7,9,11,15-pentahydroxy hexacos-2-enoic acid; ^{1}H- and ^{13}C-NMR analysis; mass spectra; salvigenin; cirsimaritin.

Abstract—Isolation and structure determination of the δ-lactones of *Z*-5,7,9,11-tetrahydroxy- and *Z*-5,7,9,11,15-pentahydroxyhexacos-2-enoic acids from *Eupatorium pilosum* is reported. The flavones salvigenin and cirsimaritin were also found.

INTRODUCTION

In the present paper we continue our reports on the constituents of *Eupatorium* species *sensu stricto* which have produced a number of cytotoxic and antitumor sesquiterpene lactones [1, 2]. Here we describe the isolation and structure determination of two unusual fatty acid derivatives **1a** and **2a** from *Eupatorium pilsoum* Walt. Two relatively rare flavones, salvigenin and cirsimaritin, were also found.

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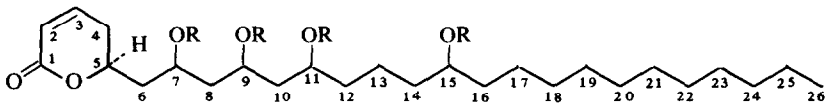
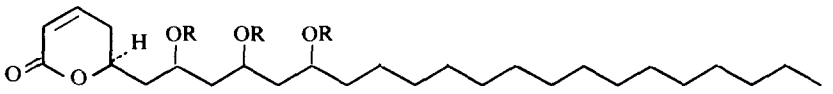
RESULTS AND DISCUSSION

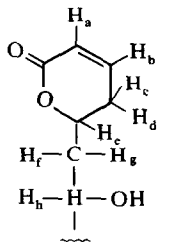
Substance **1a**, $C_{26}H_{48}O_6$ by elemental analysis and chemical ionization MS (in the high resolution electron impact MS the peak of highest *m/e* corresponded to formula $C_{26}H_{44}O_4$ due to loss of two molecules of H_2O), mp 109°, $[\alpha]_D$ − 33.6°, was an α, β-unsaturated δ-lactone as evidenced by an IR band at 1720 cm^{-1}, the circular dichroism ($[\theta]_{253}$ − 1950) and demonstration of partial structure **3** by NMR spectrometry as follows.



1a R = H
1b R = Ac



2a R = H
2b R = Ac



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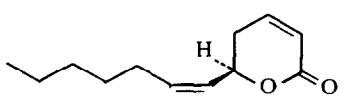

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Signals of H_a (broadened *d*) and H_b (*m*) were located at δ 6.02 and 6.91, respectively (Table 1). Irradiation at the frequency of H_a simplified the signal of H_b; irradiation at the frequency of H_b collapsed H_a to a broadened singlet and simplified a two proton multiplet at 2.42 ppm (H_c and H_d). Conversely, irradiation at the latter frequency sharpened H_a, collapsed the signal of H_b to a doublet ($J_{a,b}$ = 10 Hz), and simplified a multiplet at 4.63 ppm (H_e) to *dd* ($J_{e,f}$ = 7, $J_{e,g}$ = 5 Hz). Further irradiation at the frequency of H_e affected multiplets at 2.04 (H_f) and 1.77 ppm (H_g). Irradiation at the frequencies of H_f and H_g simplified not only the multiplet of H_e, but also a signal at 4.14 ppm associated with two protons on carbon carrying a secondary hydroxyl.

Acetylation of **1a** resulted in formation of a tetra-acetate **1b**; this was accompanied by a downfield shift of the two proton signal at 4.14 and two OMe proton signals at 3.89 and 3.58 ppm, thus indicating the presence of four secondary hydroxyl groups. These conclusions were substantiated by the ^{13}C-NMR spectrum (Table 2) which exhibited five doublets in the range characteristic of >CH-O. Irradiation at the frequencies of the methine protons did not disturb any signals below 3 ppm, but affected only the methylene region. Hence a vicinal glycol system was absent, as was also inferred from a negative periodate test.

Since the three degrees of unsaturation demanded by the molecular formula were met by the unsaturated δ-lactone of partial structure **3** and since the NMR spectra showed that the substance contained only one methyl group it followed that the remaining 18 carbon atoms required by the empirical formula had to be arranged in a straight chain bearing three additional secondary hydroxyl groups. The locations of these hydroxyl groups were deduced by analysis of the ^{13}C-NMR and high resolution MS of **1a** and those of a second lactone (**2a**) isolated from *E. pilosum*. This substance, mp 101°, $C_{26}H_{48}O_5$ (elemental analysis and chemical ionization MS; again the molecular ion was absent from the high resolution electron impact MS), had one less hydroxyl group than **1a** (^{13}C-NMR spectrum, formation of a triacetate). The presence in **2a** of partial structure **3** and the absence of a vicinal glycol system was demonstrated in the manner previously detailed for **1a**.

Chemical shifts of the methinyl and methylene signals in the ^{13}C-NMR spectrum of the trihydroxy lactone (Table 2) permitted expansion of its partial structure **3** to **2a**. The triplets at 22.69 and 31.92 ppm were easily identifiable as arising from C-25 and C-24, respectively [3–5]; that the signal of C-3 was in the cluster near 29 ppm was evident from recently-published work on argentilactone (**4**) [6]. Use of chemical shift parameters [3–5] indicated that the substance contained one methylene group γ to a hydroxyl (triplet near 25 ppm), one methylene β to one hydroxyl (triplet near 38 ppm) and three methylenes each β to two hydroxyls or one hydroxyl and one lactone oxygen (triplets near 43 ppm). Single frequency off-resonance decoupling demonstrated that the doublet at 76.13 ppm was due to C-5. While selective decoupling of C-6 was not possible, any arrangement of hydroxyl groups other than that of formula **2a** (with C-9 being assigned the methinyl frequency at higher field, 69.23 ppm, because of the γ-effect of two hydroxyl groups) violated the requirements specified above.

The ^{13}C-NMR spectrum of the tetrahydroxy lactone was very similar to that of **2a** but exhibited one new methinyl resonance near 72 ppm, three triplets near 43 ppm characteristic of methylene β to two hydroxyls, two additional triplets near 37 ppm characteristic of methylene β to one hydroxyl and one new triplet at 21.30 ppm. The number of partially-superimposed triplets near 29 ppm was reduced to seven. By comparison with the ^{13}C-NMR spectrum of **2a**, the additional signals

Table 1. ^{1}H-NMR spectra of lactones and their derivatives*

Compound	H-2	H-3	H-4	H-5	H-6	>CHOR	—CH$_2$†	H-26‡	Misc.
1a	6.02 *dt* (10, 3)	6.91 *ddd* (10, 5, 4)	2.42 *m*†	4.63 *m*	2.04 *dt* (14, 7) 1.77 *ddd* (14, 5, 3.5)	4.14 *m* 3.89 *tbr* (4.5) 3.58 *m*	1.56 *m* 1.52 *m* } 12H 1.23 *c*-18 H	0.87 *t* (7)	4.02 3.38 (—OH)
1b	6.01 *ddd* (10, 2, 1)	6.89 *ddd* (10, 6, 2)	2.43 *ddd* (18, 6, 4, 1) 2.30 *ddt* (18, 11, 2)	4.48 *m*		5.05 *m* 4.94 *q*(6) 4.87 *q*(6)	2*c* 1.5 *m* } 14H 1.24 *c*-18 H	0.88 *t* (7)	2.07, 2.06 2.05, 2.03 (Ac)
2a	6.02 *dt* (10, 3)	6.91 *ddd* (10, 5, 4)	2.44 *m*†	4.68 *m*	2.04 *ddd* (14, 8, 7) 1.77 *ddd* (14, 6, 4)	4.14 *m*† 3.86 *m*	1.5 *c*-10 H 1.25 *c*-22 H	0.88 *t* (7)	4.43, 3.97, 2.72 (—OH)
2b	6.01 *ddd* (10, 2, 1)	6.86 *ddd* (10, 6, 2)	2.43 *dddd* (18, 6, 4, 1) 2.29 *dddd* (18, 11, 2, 2)	4.48 *m*		5.04 *m* 4.95 *q*(6) 4.90 *q*(6)	2 c 1.67 *m* 1.51 *m* } 12H 1.26*c*-22 H	0.88 *t* (7)	2.07, 2.06 2.05 (Ac)

* Run in $CDCl_3$ at 270 MHz. Unmarked signals are singlets. c is a complex signal whose centre is given. Values in parentheses are coupling constants.

† Integration of methylene region carried out in solutions containing shift reagent.

‡ Intensity three protons.

§ Intensity two protons.

¶ Submerged in —CH_2 region.

Table 2. ^{13}C-NMR spectra of **1a** and **2a***

Signal	1a	Assignment	2a	Assignment
1	164.30	C-1	164.10	C-1
2	145.47 *d*	C-3	145.39 *d*	C-3
3	121.15 *d*	C-2	121.17 *d*	C-2
4	76.18 *d*	C-5†	76.13 d	C-5
5	73.27 *d*‡	C-7	73.62 *d*	C-7
6	72.67 *d*§	C-11	73.21 *d*	C-11
7	71.85 *d*¶	C-15	69.29 *d*	C-9
8	69.13 *d*‡	C-9	43.42 *t*	C-6, C-8
9	43.50 *t*‖	C-6, C-8	42.19 *t*	C-10
10	42.27 *t*	C-10	38.40 *t*	C-12
11	38.08 *t*	C-12	31.92 *t*	C-24
12	37.64 *t*	C-14	29.69 *t*	C-4
13	37.04 *t*	C-16	29.39 *t*	C-14
14	31.92 *t*	C-24	28.38 *t*	through C-23
15	29.66 *t***	C-4	25.30 *t*	C-13
16	29.44 *t*	C-18	22.69 *t*	C-25
17	29 36 *t*‖	through C-23	14.14 *q*	C-26
18	25.77 *t*	C-17		
19	22.69 *t*	C-25		
20	21.30 *t*	C-13		
21	14.11 *q*	C-26		

* Run in $CDCl_3$ at 67.905 MHz. Unmarked signals are singlets.
† Identified by single frequency off resonance decoupling.
‡ Collapsed by irradiation at 4.14 ppm.
§ Collapsed on irradiation at 3.89 ppm.
¶ Collapsed on irradiation at 3.58 ppm.
‖ Two superimposed signals.
** Four superimposed signals.
†† Eleven carbons by difference.

could be reconciled only with formula **1a** where the triplet at 21.30 ppm is assigned to C-13 because of the γ-effect of two hydroxyl groups.

Formulas **1a** and **2a** were supported by the high resolution MS (Schemes 1 and 2) which contained peaks characteristic of fragmentation modes *a–h* and *a–f*, respectively, due to α-cleavage with respect to the carbon atoms carrying the hydroxyl groups [7, 8]. Relatively abundant ions were observed of composition $C_5H_8O_2$ and C_5H_5O characteristic [7] of fragmentation *i* (or *g* in the case of **2a**) followed by loss of oxygen and ring contraction. Of particular significance were the two sets of ions arising by fragmentation modes *d* and *f* in the case of **1a** and *b* and *d* in the case of **2a**. Each set accounts for all carbon atoms of the molecule; the composition of the fragment ions requires that hydroxyl groups be attached to C-9 and C-11. The location of the extra hydroxyl group of **1a** was not as readily apparent but may be inferred from formation of the ions arising from scission mode *a*, which must derive by loss of water from an unobserved ion $C_{15}H_{25}O_6$, and from observation of a weak ion $C_{12}H_{25}O$ arising by scission mode *b*.

Application of rules governing the relationship between Cotton effect and absolute configuration of pentenolides indicates that the absolute configuration at C-5 of **1a** and **2a** is *R* as shown in the formulas if it is assumed that the C-5 side chain is equatorial [9, 10]. The stereochemistry at the remaining centers remains unknown.

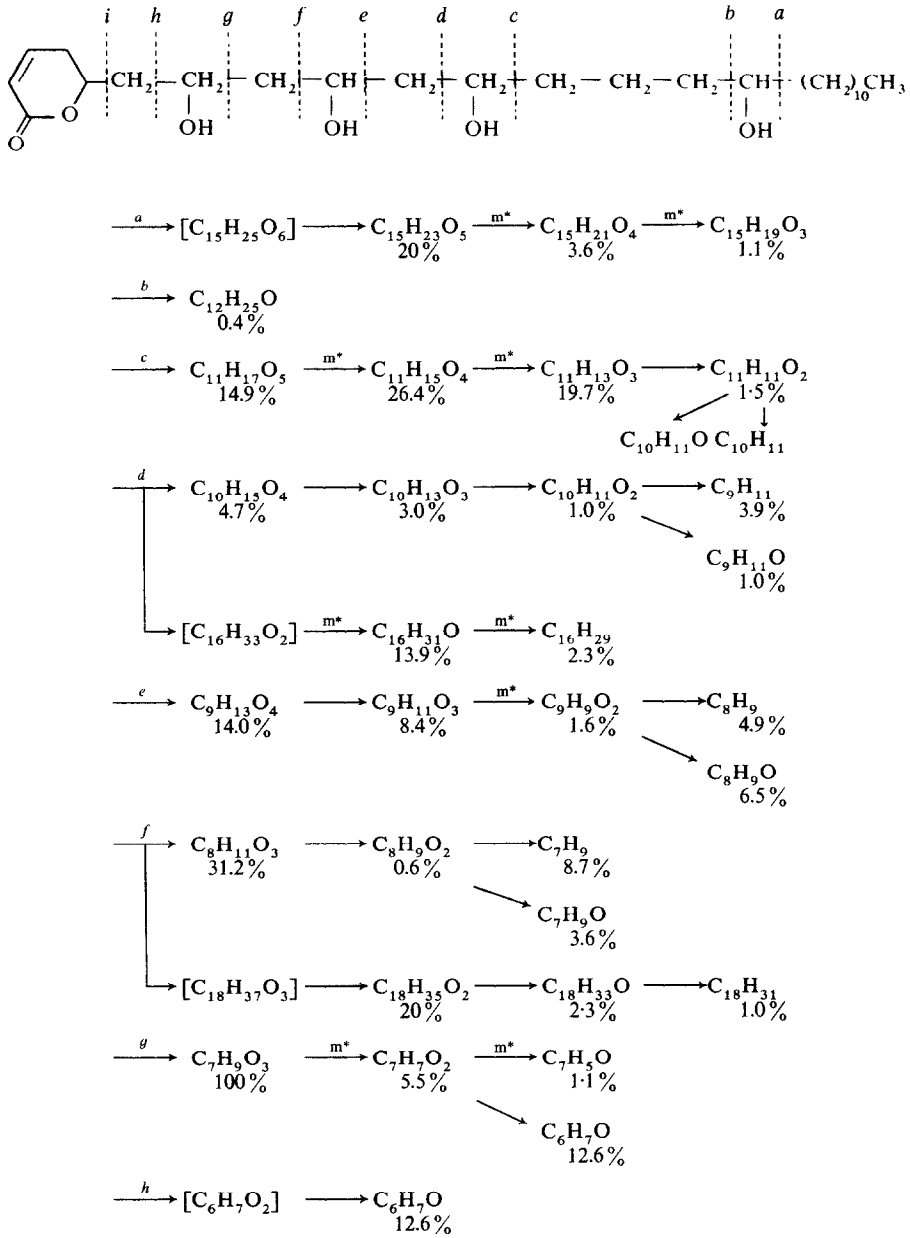
Compounds **1a** and **2a** are higher homologs of a series of naturally-occurring C-12 α, β-unsaturated δ-lactones [6]. γ-Lactones of long chain fatty acids occur as mixtures in certain plant waxes [11] and the isolation of the lactone of δ-hydroxyhexacosanoic acid has been reported recently [12]. The position of the functional groups along the chain suggests that biosynthesis of **1a** and **2a** may involve at least partial retention of the oxygen atoms which are introduced during the various stages of chain elongation rather than desaturation of hexacosanoic acid followed by hydration.

EXPERIMENTAL

Extraction of Eupatorium pilosum. Above ground parts of *E. pilosum* Walt., collected by Dr. R. K. Godfrey on August 4, 1968 along U.S. 98 one half mile northeast of Lanark, Franklin County, Florida (Godfrey #67976 on deposit in herbarium of Florida State University), wt. 16.5 kg, was extracted with $CHCl_3$ and worked up in the usual manner [13]. The dark brown gum (61 g) was adsorbed on 0.2 kg Si gel (Mallinckrodt 100 mesh) and was chromatographed on a column of 1.1 kg Si gel packed in C_6H_6 and eluted with solvents of increasing polarity, 1 l. fractions were collected: Fr. 1–5 C_6H_6, 5–10 C_6H_6–$CHCl_3$ (1:1), 11–14 $CHCl_3$, 15–21 $CHCl_3$–MeOH (19:1), 22–26 $CHCl_3$–MeOH (9:1), 27–30 $CHCl_3$–MeOH (4:1).

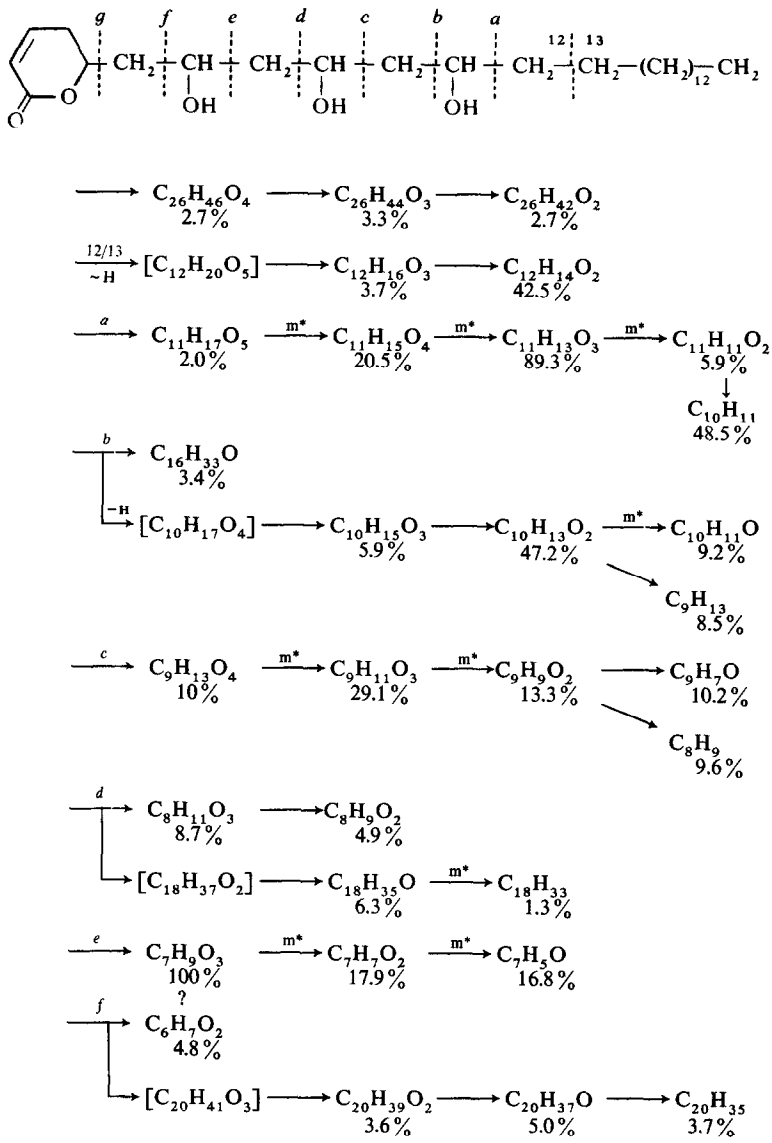
Fractions 22–26 gave a gum which on TLC exhibited a main spot at R_f 0.5 ($CHCl_3$–MeOH 9:1). Trituration with dry Et_2O resulted in partial solidification. The solid was taken up in MeOH, treated with charcoal, filtered and the filtrate was allowed to evap. to very small vol. This deposited 0.588 g of **1a** as colourless needles, mp 109°, $[\alpha]_D^{22}$ − 33.5° (*c* 0.0250, MeOH), CD curve $[\theta]_{300}$ 0, $[\theta]_{253}$ − 1950, $[\theta]_{223}$ − 2460 (last reading); UV λ_{max}^{MeOH} nm: 208 (ε 9300), IR ν_{max} cm^{-1}: 3440 (OH), 1720 (conjug. δ-lactone), 1435, 1385, 1250 and 1205. (Calcd for $C_{26}H_{48}O_6$: C, 68.38; H, 10.59; MW, 456. Found: C, 68.49; H, 10.49%; MW by CI–MS, 456). The low and high resolution MS exhibited the highest mass peak at *m/e* 420 (4.3%) (Calcd for $C_{26}H_{44}O_4$: MW 420.3238, Found: MW, 420.3230).

Scheme 1. Mass spectral fragmentation of compound **1a***



* Formulas in parentheses represent unobserved ions. The symbol m* indicates observation of a metastable peak.

Scheme 2. Mass spectral fragmentation of compound **2a**



Fractions 15–21 were combined and evap. to give 55 g highly coloured gum which was adsorbed on 100 g Si gel and re-chromatographed on 0.7 kg Si gel (J. T. Baker 60–200 mesh) 1 l. fractions being collected as follows. Fr. 1–2 hexane, fr. 3–6 hexane–Et_2O (4:1), 7–14 hexane–Et_2O (7:3), 15–17 hexane–Et_2O (1:1), fr. 18–28 Et_2O, fr. 29–32 Et_2O–MeOH (19:1), 33–36 Et_2O–MeOH (9:1). Fractions 18–22 were combined. Trituration with Et_2O and filtration of the solid furnished a mixture of two flavonoids which were separated by preparative TLC (Si gel, $CHCl_3$–MeOH 9:1). The less polar material, (261 mg) had mp 186°, IR ν_{max}^{KBr} cm^{-1}: 3150, 1660, 1615, 1600, 1580, 1510, 1475, 1380, 1270, 1135 and 840, UV λ_{max}^{MeOH} nm: 330, 277, λ_{max} nm: 357, 302 and 290 after addition of $AlCl_3$; NMR (DMSO-d_6): δ 12.88 (bonded-OH, D_2O exchanged), 8.05 and 7.11 (2H each, *d*, AB system, J = 9 Hz, H-2′, H-3′, H-5′ and H-6′), 6.93 and 6.91 (H-3 and H-8), 3.93, 3.86 and 3.74 ppm (methoxyls), MS *m/e*: 328 (M^+), 313, 299, 285, 181 and 153. Direct comparison with authentic salvigenin, mp 188–189°, established identity. The monoacetate had mp 170°, lit mp 168–169° [14].

The more polar flavone was recrystallized from Me_2CO–hexane, yield 178 mg, mp 259°, IR ν_{max}^{KBr} cm^{-1}: 3150, 1670, 1610, 1575, 1370, 1270, 1190, 1135 and 840, UV λ_{max}^{MeOH} nm: 330, 227; λ_{max} nm: 390, 277 after addition of NaOMe, 380, 340 (sh), 275 after addition of sodium acetate, 360, 302 and 287 after addition of $AlCl_3$; NMR (DMSO-d_6): δ 12.93 (—OH, D_2O exchanged), 7.95 and 6.93 (2H each, *d* AB system, J = 9 Hz, H-2′, H-3′, H-5′ and H-6′), 6.91 and 6.89 (H-3 and H-8), 3.91 and 3.73 (methoxyls), MS *m/e*: 314 (M^+), 299, 271, 181 and 153. The spectral data were in good agreement with those reported for cirsimaritin, lit mp 255–257° [15], 266–268 [16]; an authentic sample could not be obtained for direct comparison. A diacetate, prepared in the usual manner and recrystallized from Me_2CO–hexane, had mp 181–182°, IR ν_{max}^{KBr} cm^{-1}: 1770, 1765, 1648, 1618, 1462, 1430, 1388, 1210, 1172, 1120, 1098 and 858, NMR ($CDCl_3$): δ 7.83 and 7.20 (2H each, *d*, AB system, J = 9 Hz), 6.86 and 6.51 (H = 3 and H-8), 3.96 and 3.83 (methoxyls), 2.46 and 2.31 ppm (acetates), MS *m/e*: 398 (calcd 398).

Fractions 22–26 from the rechromatogram were combined. Trituration with dry Et_2O furnished a solid (**2a**) which was

recrystallized from MeOH, yield 125 mg, mp 101°, CD curve $[\theta]_{300}$ 0, $[\theta]_{252}$-4360, $[\theta]_{232}$-2380, $[\theta]_{226}$-3220 (last reading); IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3460 (—OH), 1725 (conjug. δ-lactone), 1480, 1450, 1410, 1270 and 1125, UV λ_{max}^{MeOH} nm: 207 (ε 7800).

(Calcd for $C_{26}H_{48}O_5$: C, 70.86; H, 10.98; MW, 440. Found: C, 70.90: H, 10.85%; MW by CI − MS, 440). The low and high resolution MS exhibited the last peak at *m/e* 422 (27%) (Calcd. for $C_{26}H_{46}O_3$: MW, 422.3395. Found: MW, 422.3365.

Acetylation of **1a** *and* **2a**. A soln of 0.025 g **1a** in 1 ml dry C_5H_5N and 0.5 ml Ac_2O was left overnight at 4–5° and evap. *in vacuo*. The residual gum was purified by PLC (Si gel, $CHCl_3$–MeOH 9:1) to give a quantitative yield of **1b** as a gum which had IR ν_{max}^{Film} cm^{-1}: 1735, 1730, 1372 and 1242. The low resolution MS did not contain the parent peak, but like **1a** exhibited the last peak at *m/e* 420.

(Calcd for $C_{34}H_{56}O_{10}$: C, 65.36; H, 9.03; MW, 624. Found: C. 65.09; H, 8.98%; MW by CI − MS, 624.)

Acetylation of 0.025 g of **2a** in the same manner and purification by PLC gave a gum which had IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 1750, 1741, 1379, 1260 and 1060. The low resolution MS did not exhibit the molecular ion. (Calcd for $C_{32}H_{54}O_8$: C, 67.81; H, 9.60. Found: C, 67.53; H, 9.50%.)

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