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FURTHER NEW REARRANGED LANOSTANOIDS FROM THE SEEDS OF ABIES MARIESII AND A. FIRMA

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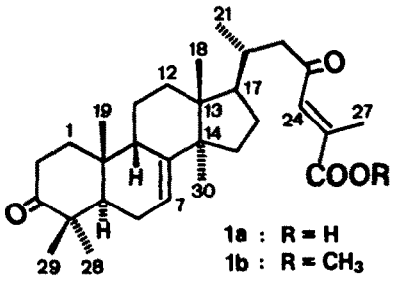
Abstract - In addition to reported 17,14-friedolanostane type (I) of triterpene further triterpenes having new rearranged lanostane skeletons, 17,13-friedolanostane (II) and 8(14→13R)abeo-17,13-friedolanostane (III), have been isolated from the seed of Abies mariesii. The skeletons I and II were also found to occur in A. firma. Their structures have been elucidated by spectral and X-ray diffraction analyses.

In the preceding papers, we reported the occurrence of an antimicrobial triterpene, mariesiic acid A (5a),[1] with an unusual skeleton, 17,14-friedolanostane (I), in the seed of Abies mariesii Mast., and of several normal lanostane triterpenes, firmanoic acid (1a), isofirmanoic acid (2a) and firmanolides (3 and 4),[2] in the seed of A. firma Sieb. et Zucc. Further detailed search for both the seed extracts led to the isolation of five new rearranged lanostanoids, named mariesiic acid B (6a), mariesiic acid C (7a) and isomariesiic acid C (8a) from A. mariesii and 23-oxo-mariesiic acid A (9a) and 23-oxo-mariesiic acid B (10a) in common. In addition, 1a and 2a were also found to occur in A. mariesii. In the present paper, we describe the structural elucidation and biogenesis of these new triterpene acids along with their antimicrobial activities.

Ether extract (yield ca 30%) from the seed of A. mariesii comprised acidic components (22.8% of the extract), from which new triterpene acids were chromatographycally purified as their methyl esters (6b-10b) after treatment with diazomethane.

Methyl mariesiate B (6b) was analyzed by mass spectrum to have the molecular formula $C_{31}H_{48}O_4$ and its IR and ^{13}C NMR (Table 2) spectra showed the presence of an α,β-unsaturated ester carbonyl (1705 cm^{-1}; 168.6 ppm) and two secondary hydroxyl groups [3600 and 3450 cm^{-1}; 66.9 (d) and 76.5 (d) ppm] in common with the case of 5b. The ^{13}C NMR spectrum showed also six sp^2 carbon signals due to three double bonds suggestive of tetracarbocyclic nature for this triterpene. The ^1H NMR spectrum (Table 1) revealed signals of two methine protons (δ 3.46 and 4.50) geminating to the hydroxyl groups, three trisubsutituted olefinic protons (δ 5.48, 5.63 and 6.70) and seven C-methyl groups ascribed to five tertiary, one secondary and one vinylic methyls. The above proton signals were very similar to those of 5b except for the chemical shifts of two tertiary methyl groups and one olefinic proton [δ 5.48 for 6b; δ 5.18 for 5b], and thus 6b involved 3α-hydroxyl group, C-7



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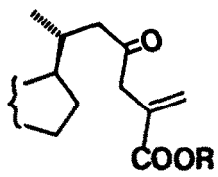
1b : R = CH_3



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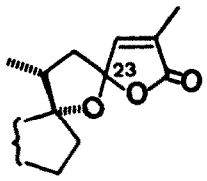

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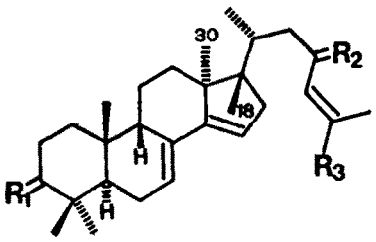
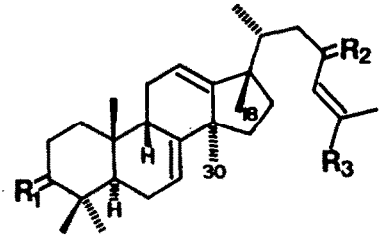
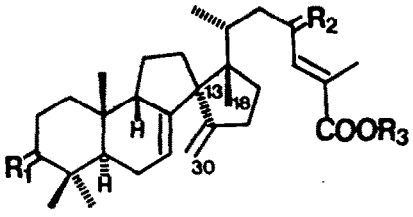
4 : 23R



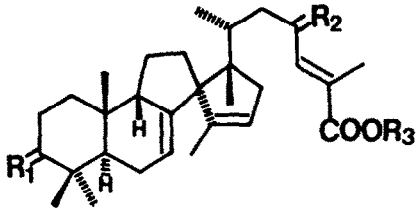
	R_1	R_2	R_3
5a	H, OH	OH, H	COOH
5b	H, OH	OH, H	$COOCH_3$
12	H, OH	OH, H	CH_2OH
9a	H, OH	=O	COOH
9b	H, OH	=O	$COOCH_3$
21	=O	=O	$COOCH_3$



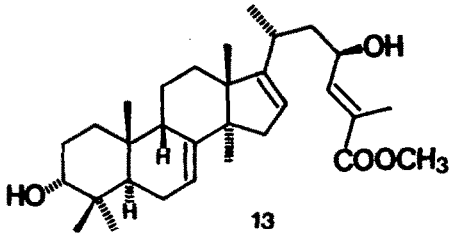
	R_1	R_2	R_3
6a	H, OH	OH, H	COOH
6b	H, OH	OH, H	$COOCH_3$
11	H, OH	OH, H	CH_2OH
10a	H, OH	=O	COOH
10b	H, OH	=O	$COOCH_3$
22	=O	=O	$COOCH_3$



	R_1	R_2	R_3
7a	=O	=O	H
7b	=O	=O	CH_3
14	OH, H	H, OH	CH_3
15	OH, H	OH, H	CH_3



	R_1	R_2	R_3
8a	=O	=O	H
8b	=O	=O	CH_3
16	OH, H	H, OH	CH_3
17	OH, H	OH, H	CH_3



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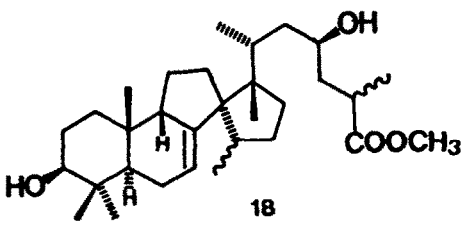

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double bond and the same side chain as that of 5b. The absence of absorption maximum above 202 nm in the UV spectra of 6b as well as the triol 11 derived from 6b by reduction with lithium aluminum hydride showed that two double bonds in the ring were unconjugated each other, in contrast to 5b [5b: λ_{max} 219 nm; the triol 12: λ_{max} 226 nm]. Therefore, two biogenetically conceivable structures, 6b and 13, were supposed for methyl mariesiate B. In the ^{1}H shift-correlated 2D-NMR spectrum, the olefinic proton signal at δ 5.48 (dd, J=8.4 and 2.4 Hz) was observed to correlate with signals at δ 2.22 (ddd, J=14.4, 8.4 and 3.4 Hz) and δ 1.83 (ddd, J=14.4, 12.0 and 2.4 Hz) assigned to allylic methylene protons. In addition, the allylic methylene signals were shown to correlate with a proton signal at δ 1.41 ascribed to 9β-H, shielded by the anisotropic effect of C-12 double bond, supporting the structure 6b for the compound. This was also in consistent with the fact that the largely different coupling constants between the olefinic proton and each of the allylic methylene protons were observed indicating the location of the double bond in the six-membered ring. Consequently, the strucutre of methyl mariesiate B was deduced as methyl (24E)-3α,23R-dihydroxy-17,13-friedo-9β-lanosta-7,12,24-trien-26-oate, 6b.

Table 1. ^{1}H NMR spectral data for 5b-10b (400 MHz, $CDCl_3$, J and $W_{1/2}$/Hz)

	5b	9b	6b	10b	7b	8b
3-H*	3.46 m	3.49 m	3.46 m	3.49 m		
7-H	5.57 br d	5.58 br d	5.63 br d	5.65 br d	5.44 dt	5.23 dt
	J=4.2	J=4.9	J=5.4	J=5.9	J=7.3, 2.2	J=7.6, 2.0
12-H			5.48 dd	5.49 dd		
			J=8.4, 2.4	J=8.3, 2.2		
15-H	5.18 dd	5.20 dd				5.27 m
	J=2.9, 1.2	J=2.9, 1.2				$W_{1/2}$=6.0
21-H†	0.94 d	0.85 d	0.98 d	0.85 d	0.84 d	0.69 d
23-H†	4.57 ddd		4.50 ddd			
24-H†	6.71 dq	7.09 q	6.70 dq	7.06 q	7.02 q	7.07 q
27-H†	1.87 d	2.22 d	1.87 d	2.22 d	2.22 d	2.21 d
angular	1.00, 0.97	1.00, 0.98	1.17, 0.98	1.21, 0.99	1.12, 1.10	1.19, 1.11
methyls	0.94, 0.93	0.95, 0.90	0.94, 0.93	0.93, 0.93	1.04, 0.81	1.08, 0.95
	0.86	0.90	0.93	0.93		
30-H					4.70 m, 4.56 m	1.43 m
					$W_{1/2}$=4 $W_{1/2}$=6	$W_{1/2}$=5
OCH_3	3.76	3.82	3.75	3.81	3.81	3.81

* $W_{1/2}$=7 Hz; † $J_{20,21}$=6.6 Hz, $J_{22,23}$≒10 Hz, $J_{22',23}$≒3 Hz, $J_{23,24}$≒8 Hz, $J_{24,27}$=1.4 Hz.

Methyl mariesiate C (7b) had the molecular formula $C_{31}H_{44}O_4$ and its IR spectrum indicated strong absorption bands due to a methoxycarbonyl (1730 and 1255 cm^{-1}), two ketone carbonyl (1710 and 1700 cm^{-1}) and an exocyclic methylene (885 cm^{-1}) groups. In analogy with the case of methyl firmanoate (1b), the presence of a partial structure, $-COCH=C(CH_3)COOCH_3$, in the side chain was revealed by its mass fragment ion at m/z 127 ($C_6H_7O_3$), UV absorption maximum at 235 nm and proton signals [δ 7.02 (1H, q, J=1.5 Hz), 2.22 (3H, d, J=1.5 Hz) and 3.81 (3H, s)] and, on biogenetic consideration, another carbonyl group was supposed to be located at C-3. The ^{1}H NMR spectrum (Table 1) showed proton signals of another trisubstituted double bond (δ 5.44), an exocyclic methylene group (δ 4.56 and 4.70) and four tertiary and one secondary methyl groups. The presence of the secondary methyl

Table 2. ^{13}C NMR spectral data (25.1 MHz, $CDCl_3$)

C	5b	9b	21*	6b	10b	22*	7b	8b
1	29.2	28.7	35.6	29.7	29.4	36.2	36.3	36.6
2	25.3	25.3	35.0	25.2	25.4	35.1	34.9	34.9
3	76.4	76.5	216.2	76.5	76.5	216.4	216.3	216.3
4	37.1	37.1	47.3	37.0	37.0	47.2	47.5	47.4
5	37.9	38.0	44.9	37.9†	38.0	45.2	48.0	46.9
6	23.1	23.1	23.9	23.2	23.2	23.9	24.4	24.0
7	120.4	120.8	120.2	122.0	122.5	121.8	123.8	122.6
8	136.8	136.6	136.7	146.2	146.0	146.2	144.8	145.5
9	53.1	52.9	52.4	51.1	51.0	50.4	58.2	55.6
10	34.7	34.8	34.7	34.8	34.8	34.8	34.9	34.6
11	33.5	33.5	33.3	28.2	28.1	27.7	24.6†	26.1†
12	25.3	25.3	25.0	118.6	118.7	118.4	27.7†	27.0†
13	51.7	51.7	51.7	156.4	156.0	156.2	61.4	63.1
14	153.3	152.8	152.0	50.0	50.0	49.8	160.8	146.5
15	114.7	115.0	115.7	36.7	36.8	36.9	33.0††	118.7
16	45.4	45.0	44.9	38.1†	38.4	38.1	33.5††	38.4
17	50.8	50.5	50.5	46.8	46.4	46.5	49.1	51.2
18	16.7	16.6	16.6	25.2	24.8	24.9	16.8	22.3
19	22.3	22.3	22.6†	22.2	22.2	22.6†	22.4	22.3
20	33.5	34.0	33.9	38.1	38.7	38.6	31.1	35.3
21	19.0	19.2	19.2	15.4	15.8	15.8	17.2	19.7
22	39.5	48.6	48.6	39.3	48.6	48.4	49.0	49.0
23	66.6	202.0	201.7	66.9	202.4	202.1	201.9	202.1
24	145.3	133.0	132.9	144.9	133.0	132.9	133.1	132.9
25	126.4	140.0	140.1	126.8	139.8	139.8	139.9	140.1
26	168.6	168.1	168.0	168.6	168.2	168.0	168.1	168.1
27	12.6	14.3	14.3	12.7	14.3	14.2	14.4	14.3
28	28.3	28.2	25.5	28.2	28.1	25.5	26.0	25.7
29	23.1	23.1	21.6†	23.0	23.0	21.5†	22.8	22.7
30	15.4	17.1	17.1	26.1	25.8	25.9	104.5	13.2
OMe	51.8	52.5	52.4	51.9	52.5	52.4	52.6	52.5

* For assistance of carbon assignment methyl 3,23-dioxo-mariesiates A (21) and B (22) were prepared.
†, †† Assignments may be interchanged.

group ascribable to 21-CH_3 and the lack of an angular methyl group for the tetracyclic triterpene suggested that the exocyclic methylene group must come from the angular methyl group. Thus, methyl mariesiate C was considered to have an unusual carbon skeleton which might arise from the lanostane and the establishment of its gross structure was subjected to X-ray analysis.

The crystalline derivative 14 suited for the analysis was prepared together with the epimer 15 at C-23 from 7b by reduction with sodium borohydride. The crystals grown in a solution of chloroform-hexane were thin plates, mp 105-106 °C, solvated with water. The cell dimensions and intensity data were obtained from the measurement on a Philips PW1100 diffractometer using Cu Kα radiation monochromated with graphite plate.

Crystal data: $C_{31}H_{48}O_4 \cdot H_2O$, FW=502.7, monoclinic, space group $P2_1$, a=12.208(8), b=17.445(10), c=6.967(4) Å, β=105.40(5)°, V=1430.5 Å^3, Z=2, D_c=1.167 g cm^{-3}, μ for Cu Kα=5.7 cm^{-1}.

Intensities of 2594 reflections out of 2953 theoretically possible ones were measured as above the 2σ(I) level in the 2θ range 6° through 156°. The crystal structure was determined by the direct method based on MULTAN[3] procedure and refined by the block-diagonal least-squares method. All the hydrogen atoms except those attached to C-31 were found on the difference electron-density map and their atomic coordinates and isotropic temperature factors were refined. The final R value was 0.052.[4] The molecular structure was illustrated in Fig. 1 drawn by PLUTO[5] program. Accordingly, the structure of methyl mariesiate C itself was determined as methyl (24E)-3,23-dioxo-8(14→13R)abeo-17,13-friedo-9β-lanosta-7,14(30),24-trien-26-oate represented by the chemical structure 7b.

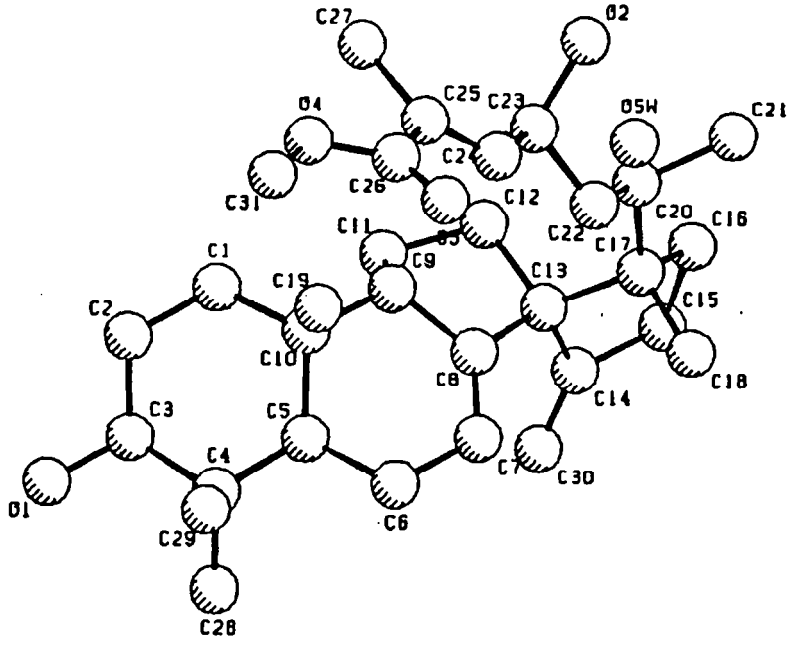
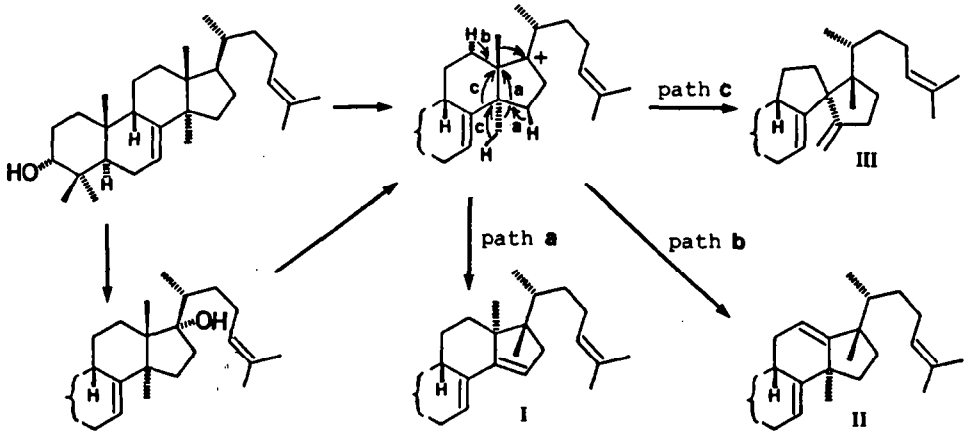


Figure 1. Molecular structure of the diol 14 derived from methyl mariesiate C (7b).

Methyl isomariesiate C (8b) having the molecular formula $C_{31}H_{44}O_4$ showed a UV absorption maximum at 235 nm and IR absorption bands at 1720, 1700 and 1695 cm^{-1} in common with the case of 7b, suggesting the presense of 3-oxo group and the same side chain as that of 7b, which was ascertained by their proton and carbon signals in its NMR spectra (see Tables 1 and 2). In addition, the ^{1}H NMR spectrum showed proton signals for two trisubsituted double bonds (δ 5.27 and 5.23) and four tertiary and one vinylic (δ 1.43) methyl groups, the last of which was shown to be coupled with the olefin signal at δ 5.27 by decoupling experiment. The olefin signal at δ 5.23 appearing as double triplet (J=7.6 and 2.0 Hz) was assigned to that of C-7 double bond because the coupling pattern was almost identical to that of 7b. The above spectral features suggested that methyl isomariesiate C was an isomer (8b) of 7b in which the double bond at C-14 migrated into the ring. This was confirmed by the formations of the compound by acid catalyzed isomerization of 7b and of a mixture of four diastereoisomers at C-14 and C-25, 18, from 15 and 17, derived from 8b, upon catalytic hydrogenation.

The structures of the other two triterpenes designated methyl 23-oxo-mariesiates A [$C_{31}H_{46}O_4$, λ_{max} 232 nm (ε 12800), ν_{max} 3640, 1730, 1690 cm^{-1}] and B [$C_{31}H_{46}O_4$, λ_{max} 235 nm (ε 9500), ν_{max} 3650, 1730, 1690 cm^{-1}], were supposed to be 23-oxo derivatives corresponding to 5b and 6b mainly on the basis of their ^1H and ^{13}C NMR spectral comparisons (see Tables 1 and 2). This was chemically confirmed by conversions of 5b and 6b with manganese dioxide into 9b and 10b, respectively, identical in all respects with the natural compounds.

The new skeletons I-III of the above triterpenes were considered most likely to be biosynthesized from the lanostane skeleton by enzymic dehydrogenation of 17-H or dehydroxylation of 17-OH followed by successive 1,2-shifts of methyl group(s) and a ring bond as shown in Scheme 1, because the normal lanostane and/or its 17-oxygenated triterpens (1-4) co-occurred in both seeds.



Scheme 1. Postulated biogenesis of the rearranged lanostanoids.

As summarized in Table 3, all triterpene acids exhibited antimicrobial activity against Gram-positive bacteria and actinomycetes tested. However, their methyl esters and simple model compounds with the side chain moiety, (2E)-2-methyl-4-oxo-2-pentenoic acid (19) and its 4-hydroxyl derivative (20), were inactive at 30 μg/disc. This result suggested that not only the carboxylic moiety but also the hydrophobic group played an important role in revealing the inhibitory activity.

Table 3. Antimicrobial activity of mariesiic acids and firmanoic acid*

	5a			6a			7a	9a	10a	1a
	Concentration (μg/disc)									
Test organism	30	15	7.5	30	15	7.5	30	30	30	30
Bacillus subtilis	14	12	9	14	12	+	9	9	11	13
Micrococcus luteus	15	13	9	16	12	10	9	9	12	13
Nocardia corallina	11	10	9	9	+	-	+	+	+	+

* The inhibitory activity was represented by the diameter of clear zone using paper disc (ϕ 8 mm) after incubation at 30 °C for 21 h. All compounds tested were inactive against Gram-negative bacteria (Escherichia coli, Pseudomonas aeruginosa) and yeast (Saccharomyces cerevisiae, Candida utilis).

EXPERIMENTAL

General. Melting points are uncorrected. Optical rotations were determined on a JASCO DIP-SL automatic polarimeter. UV spectra were measured in EtOH solution with a Hitachi 320 spectrophotometer. IR spectra were obtained on a Hitachi 215 spectrophotometer. ^{1}H NMR spectra were recorded on JEOL JNM-PX 100 (100 MHz) and JEOL JNM GX-400 (400 MHz), and ^{13}C NMR spectra were obtained on the former instrument (25.1 MHz) each in $CDCl_3$ containing TMS as an internal standard. Mass spectra were operated on a Hitachi M-80 using direct insertion at 70 eV. Wakogel C-200 and LiChroprep Si 60 (Merck) were used for column chromatography. Silica gel 60 HF_{254} (Merck) and precoated silica gel 60 F_{254} were used for preparative and analytical TLC, respectively.

Extraction. Seeds of A. mariesii (900 g), collected in Nagano Prefecture, Japan, were ground and extracted with ether (2 l x 3). The combined ether extract was concentrated to ca 1 l and treated successively with aqueous 5% Na_2CO_3 and 5% NaOH (each 500 ml x 3). The ether layer was dried over Na_2SO_4 and evaporated to afford a neutral portion (210 g). Both the aqueous alkaline layers were acidified (pH 3) with 6 N HCl under cooling and extracted with ether to yield a strongly acidic (53 g) and a weakly acidic (9 g) portions. Extraction and fractionation from seeds of A. firma was described in the preceding paper.[2]

Isolation of mariesiic acids A (5a) and B (6a) from A. mariesii. The strongly acidic portion (53 g) was chromatographed on a silica gel column (36 cm x 7.5 cm i.d.) eluting with hexane-EtOAc (2 : 1, 3 l) and then hexane-EtOAc (1 : 2, 3 l), each 300 ml of the eluant being collected. The fractions 10-20 deposited almost pure crystals (ca 5 g) which were recrystallized from EtOAc to afford 5a.[1] The combined mother liquor of the fractions 10-20 gave an oil (12.5 g), a portion (1.2 g) of which was methylated with an ethereal solution of diazomethane and chromatographed on a 10% $AgNO_3$-silica gel (130 g) column (ether-$CHCl_3$, 1 : 2) to give 5b (361 mg) and 6b (182 mg).

5b: a gum; UV: λ_{max} 219 nm (ε 16300); IR ($CHCl_3$): ν_{max} 3610, 3470, 2940, 2880, 1705, 1650, 1440, 1370, 1240, 1130, 1100, 1040, 980, 900, 835 cm^{-1}; ^{1}H and ^{13}C NMR: Tables 1 and 2; MS m/z (rel. int.) 484 (M^+, 35), 313 (100), 187 (31), 170 (38), 159 (31), 135 (42), 131 (32), 109 (32), 107 (31), 43 (63), 41 (33%).

6b: a gum; UV: λ_{max} 202 nm (end absorption); IR ($CHCl_3$): ν_{max} 3600, 3450, 2940, 2870, 1705, 1650, 1440, 1370, 1240, 1120, 1050, 980, 905, 830 cm^{-1}; ^{1}H and ^{13}C NMR: Tables 1 and 2; HRMS: m/z 484.3570 (M^+, calcd for $C_{31}H_{48}O_4$: 484.3554); MS: m/z (rel. int.) 484 (M^+, 7), 466 (M^+-H_2O, 24), 315 ($C_{22}H_{35}O$,72), 313 ($C_{22}H_{33}O$, 55), 297 ($C_{18}H_{33}O_3$, 81), 295 ($C_{22}H_{31}$, 82), 173 ($C_{13}H_{17}$, 55), 170 ($C_9H_{14}O_3$, 73), 145 ($C_{11}H_{13}$, 55), 129 ($C_6H_9O_3$, 67), 123 (C_9H_{15}, 94), 121 (C_9H_{13}, 100), 97 (90), 69 (54), 41 (64%).

A solution of 6b (103 mg) in 1 M NaOH/MeOH-H_2O (9 : 1) (10 ml) was stirred at room temperature for 2 h. After acidification with 5% HCl (pH 3) the mixture was extracted with ether. The ether extract was washed with brine, dried over Na_2SO_4 and evaporated to afford 6a (69 mg) and 6b (25 mg) after purification on a silica gel column (hexane-EtOAc, 2 : 3).

6a: mp 200-202 °C (prisms from benzene), $[\alpha]_D$ - 98.7 ° (c 0.85, acetone); UV: λ_{max} 202 nm (end absorption); IR (KBr): ν_{max} 3350, 2950, 2875, 2625, 1690, 1660, 1450, 1380, 1280, 1250, 1130, 1055, 980, 945, 905, 860, 830, 800, 750 cm^{-1}; HRMS: m/z 470.3375 (M^+, calcd for $C_{30}H_{46}O_4$: 470.3398); MS: m/z (rel. int.) 470 (M^+, 8), 315 ($C_{22}H_{35}O$, 39), 313 ($C_{22}H_{33}O$, 70), 297 ($C_{18}H_{33}O_3$, 59), 295 ($C_{22}H_{31}$, 100), 283 (40), 173 ($C_{13}H_{17}$, 68), 159 ($C_{12}H_{15}$, 49), 157 ($C_{12}H_{13}$, 44), 145 ($C_{11}H_{13}$, 56), 131 ($C_{10}H_{11}$, 43), 123 (C_9H_{15}, 99), 121 (C_9H_{13}, 95), 107 (C_8H_{11}, 45), 105 (45), 93 (45), 69 (39), 41 (50%).

$LiAlH_4$ reduction of 5b and 6b. To a solution of 5b (50 mg) in dry ether (30 ml) was added $LiAlH_4$ (10 mg) and the mixture was stirred at room temperature for 30 min. After usual work-up the product was purified by preparative TLC on silica gel developing with ether to yield the triol 12 (Rf 0.21-0.32, 33 mg), mp 181-183 °C (fine needles from $CHCl_3$-hexane); UV: λ_{max} 226 nm (ε 10100); IR (KBr): ν_{max} 3270, 2980, 1440, 1380, 1360, 1050, 980, 950, 900, 795 cm^{-1}; ^{1}H NMR (100 MHz): δ 5.52 (1H, m, 7-H), 5.46 (1H, dq, J=7.0, 1.4 Hz, 24-H), 5.15 (1H, dd, J=2.0, 2.8 Hz, 15-H), 4.48 (1H, ddd, J=3.0, 8.0, 10.0 Hz, 23-H), 4.00 (2H, s, 25-H), 3.44 (1H, m, $W_{1/2}$=6.5 Hz, 3-H), 1.71 (3H, d, J=1.4 Hz, 26-H), 0.99-0.85 (CH_3 x 6); HRMS: m/z 456.3614 (M^+, calcd for $C_{30}H_{48}O_3$: 456.3605); MS: m/z (rel. int.) 456 (M^+, 7), 438 (29), 380 (28), 314 (35), 313 (100), 159 (46), 141 (53), 135 (58), 131 (41), 107 (40), 105 (38), 69 (39), 55 (77), 43 (53), 41 (45%).

On a similar treatment as above, 6b gave the triol 11, mp 184-188 °C (fine needles from EtOAc); UV: λ_{max} 199 nm (end absorption); IR (KBr): ν_{max} 3275, 2970, 1440, 1375, 1360, 1055, 975, 950, 900, 825 cm^{-1}; ^{1}H NMR (100 MHz): δ 5.64-5.38 (3H, m, 7-, 12- and 24-H), 4.42 (1H, br t, J=9

Hz, 23-H), 4.00 (2H, s, 25-H), 3.44 (1H, m, $W_{1/2}$=6.5 Hz, 3-H), 1.71 (3H, d, J=1.4 Hz, 26-H), 1.16 (3H, s, CH_3), 0.99-0.94 (CH_3 x 5); HRMS: m/z 456.3617 (M^+, calcd for $C_{30}H_{48}O_3$: 456.3605); MS: m/z (rel. int.) 456 (M^+, 0.4), 438 (20), 423 (24), 405 (15), 313 (41), 295 (71), 173 (49), 145 (48), 141 (100), 123 (97), 121 (87), 107 (48), 105 (50), 55 (94), 43 (64), 41 (51%).

Isolation of methyl firmanoate (1b), methyl isofirmanoate (2b), methyl mariesiate C (7b) and methyl isomariesiate C (8b). The combined fractions 5-9 (4.4 g) was dissolved in ether (88 ml) and methylated with an ethereal solution of diazomethane (ca 20 ml) at - 15 °C. The methyl ester was subjected to repeated column chromatography on silica gel using hexane-EtOAc (7 : 1) and benzene-ether (30 : 1) to give 7b (1.1 g), 2b (101 mg) and a mixture of 1b and 8b (595 mg). The mixture was separated by preparative TLC on 10% $AgNO_3$-silica gel (ether-$CHCl_3$, 1 : 40) to afford 1b (Rf 0.5-0.6, 485 mg) and 8b (Rf 0.6-0.7, 80 mg). The fractions 5-9 also contained a small amount (10 mg) of 9b and 10b in a ratio of ca 1 : 2, which was confirmed by comparison of the ^1H NMR spectrum and TLC behavior with those isolated from A. firma described below.

7b: a gum, $[\alpha]_D$ - 33.6 ° (c 6.17, $CHCl_3$); UV: λ_{max} 235 nm (ε 15100); IR (CCl_4): ν_{max} 2975, 2875, 1730, 1710, 1700, 1620, 1440, 1380, 1255, 1130, 1110, 1075, 885 cm^{-1}; ^1H and ^{13}C NMR: Tables 1 and 2; HRMS: m/z 480.3228 (M^+, calcd for $C_{31}H_{44}O_4$: 480.3241); MS: m/z (rel. int.) 480 (M^+, 28), 311 ($C_{21}H_{31}O$, 33), 310 (31), 309 ($C_{22}H_{29}O$, 100), 127 ($C_6H_7O_3$, 48), 119 (C_9H_{11}, 37), 91 (32), 81 (29), 69 (31), 55 (33), 41 (36%).

8b: a gum, $[\alpha]_D$ - 154 ° (c 3.18, $CHCl_3$); UV: λ_{max} 235 nm (ε 12700); IR ($CHCl_3$): ν_{max} 2950, 2860, 1720, 1700, 1695, 1620, 1440, 1380, 1265, 1220, 1115, 885 cm^{-1}; ^1H and ^{13}C NMR: Tables 1 and 2; HRMS: m/z 480.3214 (M^+, calcd for $C_{31}H_{44}O_4$: 480.3241); MS: m/z (rel. int.) 480 (M^+, 20), 338 ($C_{24}H_{34}O$, 100), 311 ($C_{21}H_{31}O$, 49), 309 ($C_{22}H_{29}O$, 30), 187 ($C_{14}H_{19}$, 61), 185 ($C_{14}H_{17}$, 61), 145 ($C_{11}H_{13}$, 34), 131 ($C_{10}H_{11}$, 51), 127 ($C_6H_7O_3$, 57), 105 (C_8H_9, 34), 91 (29), 69 (36), 55 (34), 41 (35%).

$NaBH_4$ reduction of 7b and 8b. To a solution of 7b (411 mg) in MeOH (20 ml) was added $NaBH_4$ and the mixture was stirred for 30 min. After addition of aqueous NH_4Cl, the mixture was extracted with ether. The extract was washed with brine, dried over $MgSO_4$ and evaporated. The product was chromatographed on a silica gel column (hexane-ether, 1 : 3) to yield the diols 15 (271 mg) and 14 (90 mg).

14: mp 105-106 °C (thin plates from hexane-$CHCl_3$, monohydrated form); IR (KBr): ν_{max} 3530, 3340, 2950, 2860, 1695, 1645, 1440, 1380, 1270, 1065, 880, 840, 755 cm^{-1}; ^1H NMR (100 MHz): δ 6.65 (1H, dq, J=8.8, 1.4 Hz, 24-H), 5.36 (1H, m, 7-H), 4.67, 4.56 (each 1H, narrow m, 30-H), 4.48 (1H, m, 23-H), 3.76 (3H, s, OCH_3), 3.22 (1H, dd, J=7.7, 6.3 Hz, 3-H), 1.89 (3H, d, J=1.4 Hz, 27-H), 1.07-0.81 (CH_3 x 5); ^{13}C NMR: 36.0, 27.5, 79.4, 38.5, 46.8, 23.5, 124.3, 144.8, 58.5, 35.3, 24.7, 27.6, 61.5, 162.1, 32.8, 33.7, 49.8, 16.6, 23.5, 33.0, 16.5, 40.2, 67.7, 143.5, 128.5, 168.2, 13.3, 28.9, 16.2, 104.7 (assignments: from C-1 to C-30), 52.1 (OCH_3); HRMS: m/z 484.3552 (M^+, calcd for $C_{31}H_{48}O_4$: 484.3554); MS: m/z (rel. int.) 484 (M^+, 27), 466 (18), 313 (68), 197 (79), 165 (67), 135 (72), 131 (67), 119 (75), 97 (100), 91 (67), 81 (71), 69 (67), 41 (96%).

15: mp 152-153 °C (fine needles from hexane-ether); IR (KBr): ν_{max} 3400, 2950, 2860, 1710, 1645, 1440, 1380, 1300, 1250, 1140, 1085, 1035, 1005, 875, 835, 755 cm^{-1}; ^1H NMR (100 MHz): δ 6.58 (1H, dq, J=8.0, 1.4 Hz, 24-H), 5.36 (1H, m, 7-H), 4.68, 4.59 (each 1H, narrow m, 30-H), 4.48 (1H, m, 23-H), 3.72 (OCH_3), 3.21 (1H, dd, J=7.7, 6.3 Hz, 3-H), 1.83 (3H, d, J=1.4 Hz, 27-H), 1.04-0.79 (CH_3 x 5); ^{13}C NMR: 36.0, 27.5, 79.4, 38.5, 46.7, 23.6, 123.8, 144.7, 58.1, 35.3, 24.7, 27.8, 61.5, 162.1, 32.9, 33.8, 49.6, 16.6, 23.4, 31.7, 15.4, 38.7, 66.5, 145.0, 126.7, 168.5, 12.7, 28.9, 16.1, 104.4 (assignments: from C-1 to C-30), 52.0 (OCH_3); HRMS: m/z 484.3554 (M^+, calcd for $C_{31}H_{48}O_4$: 484.3554); MS: m/z (rel. int.) 484 (M^+, 26), 466 (33), 313 (72), 135 (83), 131 (73), 119 (81), 97 (100), 91 (71), 81 (75), 69 (71), 41 (98%).

On a similar treatment as above 8b gave the diols 16 and 17 in a ratio of ca 1 : 3 after chromatography on a LiChroprep RP-8 (Rf 0.43 and 0.37, H_2O-MeOH, 15 : 85).

16: mp 115-116 °C (needles from EtOAc-hexane); IR (KBr): ν_{max} 3350, 2965, 2940, 2860, 1715, 1650, 1450, 1375, 1260, 1040, 750 cm^{-1}; ^1H NMR (100 MHz): δ 6.56 (1H, dq, J=7.6, 1.5 Hz, 24-H), 5.21 (1H, narrow m, 15-H), 5.16 (1H, m, 7-H), 4.47 (1H, m, 23-H), 3.75 (OCH_3), 3.19 (1H, dd, J=6.5, 8.0 Hz, 3-H), 1.89 (3H, d, J=1.5 Hz, 27-H), 1.43 (3H, narrow m, 30-H), 0.99 (6H, s), 0.94 (3H, s), 0.87 (3H, s), 0.75 (3H, d, J=6.5 Hz, 21-H); HRMS: m/z 484.3536 (M^+, calcd for $C_{31}H_{48}O_4$: 484.3554); MS: m/z (rel. int.) 484 (M^+, 13), 313 (26), 197 (100), 165 (51), 131 (49), 105 (37), 97 (50), 69 (42), 55 (37), 43 (47), 41 (61%).

17: mp 105-107 °C (needles from ether-hexane); IR (KBr): ν_{max} 3400, 2950, 2870, 1715, 1650, 1450, 1375, 1240, 1050, 750 cm^{-1}; ^1H NMR (100 MHz): δ 6.66 (1H, dq, J=8.0, 1.5 Hz, 24-H), 5.22 (1H, narrow m, 15-H), 5.16 (1H, m, 7-H), 4.47 (1H, m, 23-H), 3.73 (OCH_3), 3.20 (1H, dd, J=6.5, 8.0 Hz, 3-H), 1.86 (3H, d, J=1.5 Hz, 27-H), 1.44 (3H, narrow m, 30-H), 0.99 (9H, s), 0.88 (3H,

s), 0.76 (3H, d, J=6.9 Hz, 21-H); HRMS: m/z 484.3556 (M^+, calcd for $C_{31}H_{48}O_4$: 484.3554); MS: m/z (rel. int.) 484 (M^+, 10), 313 (28), 197 (100), 165 (55), 131 (58), 105 (40), 97 (58), 69 (45), 55 (38), 43 (41), 41 (62%).

Acid catalyzed isomerization of 7b into 8b. To a $CDCl_3$ solution of 7b in a NMR sample tube was added a drop of 10% HCl-MeOH. By monitoring on the ^1H NMR spectrum and TLC, 7b was pursued to be isomerized completely into 8b at room temperature in 22 h.

Catalytic hydrogenation of 15 and 17. The diol 15 (50 mg) was hydrogenated over PtO_2 (15 mg) in EtOH (3 ml) at room temperature for 17 h, while the diol 17 for five days. The major products from 15 and 17, showing the same Rf value on TLC, collected by chromatography on 10% $AgNO_3$-silica gel (EtOAc-hexane, 1 : 2) yielded a mixture of the tetrahydro derivatives 18 as a solid, which showed the following spectra: ^1H NMR (100 MHz): δ 5.30 (m, 7-H), 3.61 (OCH_3), 3.60 (m, 23-H), 3.20 (dd, J=6.5, 8.0 Hz, 3-H), 2.64 (sextet, J=7.1 Hz, 25-H), 1.20-0.69 (methyl groups); ^{13}C NMR: δ 177.8 and 177.4 (C-26), 145.1 (C-8), 120.4 and 120.1 (C-7), 79.4 (C-3), 68.9 and 68.1 (C-23), 60.2 and 57.4 (C-13), 58.8 and 57.0 (C-9), 51.7 (OCH_3), 50.1 and 49.7 (C-17), 46.1 (C-5), 45.5 (C-14), 42.9, 42.7, 42.5 and 42.4 (C-22 and C-24), 40.3 (t), 38.4 (C-4), 37.3 and 37.0 (C-25), 35.9 and 35.8 (C-1), 35.1 (C-10), 34.6 (t), 33.9 (C-20), 33.2 (t), 32.0 (t), 30.7 (t), 28.8 (C-28), 27.5 (C-2), 27.0 (t), 26.7 (t), 26.3 (t), 23.4 (C-6 and C-19), 20.2 (q), 17.6 (q), 17.5 (q), 17.3 (q), 16.9 (q), 16.0 (C-29), 15.2 and 15.0 (C-21); MS: m/z 488 (M^+), 456, 261, 260, 243, 222, 215, 135, 133, 131, 121, 119, 105, 95, 69, 55, 43, 41. Considering from the appearance of the carbon signals as double lines, assigned to C-7, C-13, C-17 and carbon atoms in the side chain in the ^{13}C NMR spectra, the tetrahydro derivatives were deduced to be composed of four diastereoisomers concerning C-14 and C-25.

Isolation of 9b and 10b from A. firma. The chromatographycally most polar fraction (3.9 g) of the acidic portion reported[2] was methylated with diazomethane at - 15 °C. A portion of the methyl ester (770 mg) was subjected to preparative TLC on 10% $AgNO_3$-silica gel developing with $CHCl_3$-ether (80 : 1) two times to give 9b (Rf 0.37-0.27, 200 mg) and 10b (Rf 0.23-0.10, 453 mg).

9b: a gum, $[\alpha]_D$ + 53.2 °(c 1.28, $CHCl_3$); UV: λ_{max} 232 nm (ε 12800); IR (CCl_4): ν_{max} 3640, 2970, 2880, 1730, 1690, 1620, 1440, 1385, 1365, 1255, 1125, 1100, 1065, 985, 905 cm^{-1}; ^1H and ^{13}C NMR: Tables 1 and 2; HRMS: m/z 482.3400 (M^+, calcd for $C_{31}H_{46}O_4$: 482.3398); MS: m/z (rel. int.) 482 (M^+, 11), 340 ($C_{24}H_{36}O$, 78), 314 (34), 313 ($C_{22}H_{33}O$, 100), 295 ($C_{22}H_{31}$, 50), 187 ($C_{14}H_{19}$, 32), 169 ($C_9H_{13}O_3$, 66), 127 ($C_6H_7O_3$, 36%).

10b: a gum, $[\alpha]_D$ - 157.4 ° (c 1.98, $CHCl_3$); UV: λ_{max} 235 nm (ε 9500); IR (CCl4): ν_{max} 3650, 2970, 2880, 1730, 1690, 1620, 1440, 1385, 1370, 1255, 1125, 1060, 985, 905 cm^{-1}; ^1H and ^{13}C NMR: Tables 1 and 2; HRMS: m/z 482.3374 (M^+, calcd for $C_{31}H_{46}O_4$: 482.3398); MS: m/z (rel. int.) 482 (M^+, 28), 467 ($C_{30}H_{43}O_4$, 36), 464 ($C_{31}H_{44}O_3$, 27), 449 ($C_{30}H_{41}O_3$, 41), 353 ($C_{23}H_{29}O_3$, 48), 313 ($C_{22}H_{33}O$, 98), 295 ($C_{22}H_{31}$, 100), 173 ($C_{13}H_{17}$, 62), 127 ($C_6H_7O_3$, 86), 123 (C_9H_{15}, 73), 121 (C_9H_{13}, 60%).

MnO_2 oxidation of 5b and 6b. A mixture of 5b (106 mg) and activated MnO_2 (2 g) in ether (20 ml) was stirred at room temperature for 30 min. After filtration followed by evaporation, the product was purified on a silica gel column (ether-$CHCl_3$, 1 : 20) to afford 9b (70 mg) identical in all respects (IR, ^1H NMR, MS and TLC) to the natural compound. On a similar treatment as above 6b gave 10b.

Jones oxidation of 9b and 10b. To a stirred solution of 9b (100 mg) in acetone (10 ml) was added dropwise Jones' reagent under cooling. After usual work-up the product was purified on a silica gel column (ether-$CHCl_3$, 1 : 50) to yield 21 (80 mg), a gum; UV: λ_{max} 231.5 nm (ε 14700); IR (CCl_4): ν_{max} 2970, 1730, 1710, 1700, 1620, 1450, 1435, 1385, 1365, 1255, 1125 cm^{-1}; ^1H NMR (400 MHz): δ 7.08 (1H, q, J=1.5 Hz, 24-H), 5.62 (1H, m, 7-H), 5.26 (1H, dd, J=1.5, 3.3 Hz, 15-H), 2.22 (3H, d, J=1.5 Hz, 27-H), 0.85 (3H, d, J=6.6 Hz, 21-H), 3.81, 1.14, 1.11, 1.10, 0.90, 0.89 (each 3H, s); ^{13}C NMR: Table 2 ; HRMS: m/z 480.3238 (M^+, calcd for $C_{31}H_{44}O_4$: 480.3241); MS: m/z (rel. int.) 480 (M^+, 9), 339 (15), 338 ($C_{24}H_{34}O$, 58), 323 ($C_{23}H_{31}O$, 14), 312 (19), 311 ($C_{22}H_{31}O$, 54), 187 ($C_{14}H_{19}$, 13), 170 (18), 169 ($C_9H_{13}O_3$, 100), 127 ($C_6H_7O_3$, 20%).

On a similar treatment as above 10b gave 22, a gum; UV: λ_{max} 234.5 nm (ε 9900); IR (CCl_4): ν_{max} 2980, 1730, 1715, 1700, 1620, 1455, 1440, 1385, 1370, 1260, 1130, 1120, 1070, 990, 905 cm^{-1}; ^1H NMR (400 MHz): δ 7.06 (1H, q, J=1.5 Hz, 24-H), 5.68 (1H, m, 7-H), 5.48 (1H, dd, J=8.4, 2.5 Hz, 12-H), 2.20 (3H, d, J=1.5 Hz, 27-H), 0.90 (3H, d, J = 6.6 Hz, 21-H), 3.80, 1.20, 1.11, 1.10, 1.09, 0.95 (each 3H, s); ^{13}C NMR: Table 2 ; HRMS: m/z 480.3227 (M^+, calcd for $C_{31}H_{44}O_4$: 480.3241); MS: m/z (rel. int.) 480 (M^+, 12), 447 ($C_{30}H_{39}O_3$, 25), 312 (25), 311 ($C_{22}H_{31}O$, 100), 201 ($C_{14}H_{17}O$, 21), 169 (23), 127 ($C_6H_7O_3$, 43), 123 (35), 121 (C_9H_{13}, 32), 107 (C_8H_{11}, 21%).

Preparation of (2E)-2-methyl-4-oxo-2-pentenoic acid (19). A mixture of acetone (2.5 ml), pyruvic acid (1 ml) and 85% phosphoric acid (2.5 ml) was refluxed for 70 h, as reported[6]. The

reaction mixture was poured into water (15 ml) and extracted with ether (30 ml x 3). The ether layer was extracted with 5% Na_2CO_3 (25 ml x 5) and the aqueous layer was acidified with 6 N HCl. After extraction with ether (50 ml x 3), the product was chromatographed on a silica gel (40 g) column (EtOAc-hexane, 2 : 3) to yield 19 (83 mg), mp 96-98 °C (prisms from $CHCl_3$-hexane); UV: λ_{max} 235 nm (ε 12800); IR (KBr): ν_{max} 3170-2800, 1715, 1660, 1610, 1365, 1220, 1135, 1020, 975, 905, 800, 740 cm^{-1}; ^1H NMR: δ 7.17 (1H, q, J=1.4 Hz), 2.35 (3H, s), 2.20 (3H, d, J=1.4 Hz), 10.3 (1H, br s, OH); HRMS: m/z 128.0480 (M^+, calcd for $C_6H_8O_3$: 128.0473); MS: m/z (rel. int.) 128 (M^+, 9), 113 (13), 110 (40), 85 (15), 82 (16), 43 (100), 41 (15), 39 (18%), together with its Z isomer (271 mg), mp 98-100 °C (lit. mp 99-100 °C).

The keto acid 19 was reduced with $NaBH_4$ in MeOH to afford (2E)-4-hydroxy-2-methyl-2-pentenoic acid (20), a gum; UV: λ_{max} 210 nm (ε 10300); IR ($CHCl_3$): ν_{max} 3620-2450, 1690, 1650, 1380, 1270, 1160, 1060, 940 cm^{-1}; ^1H NMR: δ 6.80 (1H, dq, J=8.0, 1.5 Hz), 6.10 (2H, br s, OH), 4.68 (1H, dq, J=8.0, 6.0 Hz), 1.88 (3H, d, J=1.5 Hz), 1.33 (3H, d, J=6 Hz); HRMS: m/z 115.0402 (M^+-CH_3, calcd for $C_5H_7O_3$: 115.0395); MS: m/z (rel. int) 115 (M^+-CH_3, 17), 112 (55), 97 (50), 87 (76), 69 (82), 45 (43), 43 (74), 41 (100), 39 (54%).

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