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FOUR DITERPENE ESTERS FROM *EUPHORBIA MYRSINITES**

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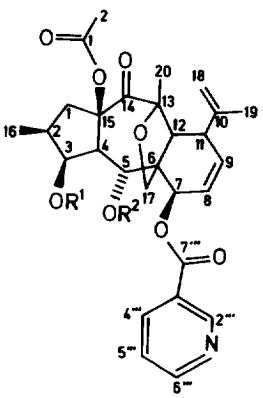
Key Word Index—*Euphorbia myrsinites*; Euphorbiaceae; tetracyclic diterpene esters; cycloartane-type triterpenoids; betulin.

Abstract—The Turkish species *Euphorbia myrsinites* has yielded four new tetracyclic diterpene tetraesters from a cytotoxic acetone extract, in addition to the known cycloartane-type triterpenoids and betulin. The new compounds and their hydrolysis product have been extensively characterized by high field spectroscopic techniques, and were shown to be four new tetraesters of the parent alcohol, myrsinol.

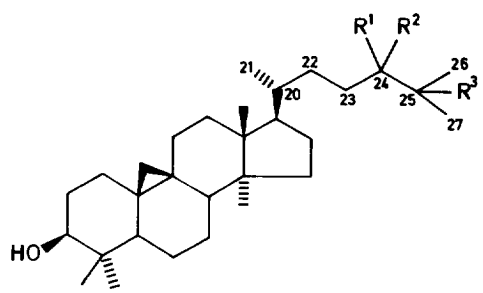
INTRODUCTION

The genus *Euphorbia* consists of about 91 species in Turkey. Euphorbiaceae plants are well known to contain irritant and tumor-promoting constituents [1], and diterpenes from Euphorbiaceae have been found to possess a number of interesting biological activities [2]. In our continuing search for biologically active compounds from the Turkish Euphorbiaceae [3, 4], we have isolated four new tetraester derivatives (**1**–**4**) of a polyfunctional diterpene alcohol, myrsinol, from *Euphorbia myrsinites* L. The plant contains substantial latex and causes severe irritation of the skin and eyes during collection. Chemical constituents of *E. myrsinites* L. have previously been investigated and the presence of ingenol-type diterpenes, as well as diterpene triesters of myrsinol have been reported [5–7]. The four tetraester derivatives of myrsinol have been characterized spectrally and chemically. Two of them, **3** and **4**, showed moderate anti HIV-1 reverse transcriptase (RT) inhibition.



1 $R^1 = R^2 = A$
2 $R^1 = A$; $R^2 = B$
2a $R^1 = A$; $R^2 = H$
3 $R^1 = B$; $R^2 = A$
4 $R^1 = R^2 = B$

A = $CO\overset{1'}{C}H_2\overset{2'}{C}H_3$ (propionyl)
B = $CO\overset{1''}{C}H_2\overset{2''}{C}H_2\overset{3''}{C}H_2\overset{4''}{C}H_3$ (butanoyl)



5 $R^1 = R^2 = CH_2$; $R^3 = H$
6 $R^1 = CH_3$; $R^2 = R^3 = H$
7 Δ^{25} ; $R^1 = OH$; $R^2 = R^3 = H$
8 Δ^{25} ; $R^1 = R^2 = O$; $R^3 = H$
9 Δ^{23} ; $R^1 = R^2 = H$; $R^3 = OH$

RESULTS AND DISCUSSION

The hexane extract of the whole plant contains a mixture of triterpenoids. Column chromatography on silica gel afforded several known triterpenoids, namely 24-methylenecycloartanol (**5**), cyclolaudanol (**6**), cycloart-25-ene-3β,24-diol (**7**), 3β-hydroxycycloart-25-en-24-one (**8**), cycloart-23-ene-3β,25-diol (**9**) and betulin (**10**).

The acetone extracts of the roots and aerial parts of *E. myrsinites* were subjected to fractionation on silica gel

*Part 3 in the series 'Biologically Active Compounds From The Euphorbiaceae'. For parts 1 and 2, see refs [3, 4].

chromatography to afford 10 fractions. Two of the more polar fractions (6 and 9) inhibited HIV-1 RT by > 99% at a concentration of 200 $\mu g\,ml^{-1}$. These two fractions were combined and separated by chromatography on silica gel to afford four tetracyclic diterpene esters **1**–**4**.

Compound **1** was obtained as a glassy gum. Its molecular formula was assigned as $C_{34}H_{41}NO_{10}$ by CI-mass spectrometry with the molecular ion peak at *m/z* 623. The IR spectrum showed no hydroxyl group, but intense absorption peaks at 1730 (ester carbonyl), 1705 (ketone), 1695, 1640 and 1595 cm^{-1} (unsaturation). The ^1H NMR spectrum (Table 1) showed three protons geminal to ester functions at δ6.10 (*d*, *J* = 11 Hz), 5.24 (*t*, *J* = 4 Hz), 5.17 (*d*, *J* = 6 Hz), for H-5, H-3 and H-7, respectively. Two olefinic protons at δ6.22 (*ddd*, *J* = 2,6,10 Hz) and 5.93 (*dd*, *J* = 6.5, 10 Hz), a pair of exocyclic methylene group protons as broadened singlets at δ4.84 and 4.80, and an acetyl group singlet at δ2.22 were observed. The spectrum also contained signals in the upfield region due to the one secondary methyl (δ0.88, *d*, *J* = 7 Hz) and two primary methyl resonances (δ1.04 and 0.95 each *t*, *J* = 7 Hz). Further analysis of the observed protons revealed the presence of one nicotinoyl, one acetyl (δ2.22) and two propionyl (*t* at δ1.04 and 0.95 and *q* at δ2.24) groups as ester functions in **1**. Through spin decoupling experiments, the sequences H_a-1, H-2, H-3, H-4 and H-5 were easily deduced. Irradiation of the signal at δ2.29 (H-4) collapsed the triplet at δ5.24 to a doublet and the doublet at δ6.10 to a singlet, indicating that these signals corresponded to H-3 and H-5, respectively. Further spin decoupling, starting with the olefinic signal at δ6.22 (H-8), led to the sequence H-7, H-9, H-11 and H-12, irradiation of H-2 (δ2.20) turned the *dd* at δ3.52 (H_a-1) to a *d* (*J* = 14 Hz), and, finally, irradiation of the *q* at δ2.24 caused the *t* at δ1.04 and 0.95 to collapse to sharp singlets indicating that this signal should be belong to the $-CH_2-$ group of a propionic acid, and the *t* at δ1.04 and 0.95 should represent the methyl groups of the propionyl moeities.

As in lathyrane-type diterpenes, the H_a-1 proton in **1** was seen at lower field (δ3.52) as *dd* (*J* = 9, 14 Hz) and the coupling constants of H_a-1, H-3 and H-5 were similar to

Table 1. ^1H NMR spectral data of compounds **1**–**4** (in $CDCl_3$, 200 MHz)

H	**1**	**2**	**2** (C_6D_6)	**3**	**4**
1a	3.52 *dd*	3.53 *dd*	3.70 *dd*	3.54 *dd*	3.52 *dd*
1b	1.44 *dd*	1.43 *dd*	1.40 *dd*	1.43 *dd*	1.42 *dd*
2	2.20 *ddd*	2.20 *ddd*	2.10 *ddd*	2.18 *t*	2.16 *t*
3	5.24 *t*	5.22 *t*	5.20 *t*	5.23 *t*	5.21 *t*
4	2.29 *dd*	2.30 *dd*	2.27 *dd*	2.30 *dd*	2.30 *dd*
5	6.10 *d*	6.09 *d*	6.29 *d*	6.08 *d*	6.09 *d*
7	5.17 *d*	5.12 *d*	5.17 *d*	5.15 *d*	5.12 *d*
8	6.22 *ddd*	6.24 *ddd*	6.15 *ddd*	6.22 *ddd*	6.22 *ddd*
9	5.93 *dd*	5.93 *dd*	5.55 *dd*	5.93 *dd*	5.93 *dd*
11	3.37 *dd*	3.37 *dd*	3.17 *dd*	3.38 *dd*	3.38 *dd*
12	3.26 *d*	3.28 *d*	3.37 *d*	3.25 *d*	3.26 *d*
16	0.88 *d*	0.89 *d*	0.73 *d*	0.89 *d*	0.89 *d*
17a	4.00 *d*	4.00 *d*	3.90 *d*	3.99 *d*	3.99 *d*
17b	3.63 *d*	3.61 *d*	3.50 *d*	3.62 *d*	3.61 *d*
18a	4.80 *br s*	4.79 *br s*	4.64 *br s*	4.81 *br s*	4.79 *br s*
18b	4.84 *br s*	4.83 *br s*	4.69 *br s*	4.84 *br s*	4.81 *br s*
19	1.75 *s*	1.75 *s*	1.72 *s*	1.76 *s*	1.76 *s*
20	1.58 *s*	1.58 *s*	1.58 *s*	1.58 *s*	1.58 *s*
OAc	2.22 *s*	2.21 *s*	1.87 *s*	2.21 *s*	2.22 *s*
Propionyl					
2′	2.24 *q*	2.18 *q*	2.24 *q*	2.18 *q*	—
3′	1.04 *t* (0.95 *t*)	1.04 *t*	1.03 *t*	0.96 *t*	—
Butanoyl					
2″	—	2.28 *t*	2.08 *t*	2.28 *t*	2.18 *t*
3″	—	1.45 *tq*	1.51 *tq*	1.44 *tq*	1.45 *tq*
4″	—	0.85 *t*	0.79 *t*	0.87 *t*	0.86 *t* (0.84 *t*)
Nicotinoyl					
2‴	9.16 *d*	9.16 *d*	9.54 *d*	9.16 *d*	9.15 *d*
4‴	8.24 *ddd*	8.24 *ddd*	8.18 *ddd*	8.23 *ddd*	8.24 *ddd*
5‴	7.39 *dd*	7.39 *dd*	6.62 *dd*	7.39 *dd*	7.39 *dd*
6‴	8.76 *dd*	8.76 *dd*	8.44 *dd*	8.76 *dd*	8.76 *dd*

J (Hz): 1a, 2α = 9; 1b,2α = 11; 1a,1b = 14; 2α,3α = 4; 3α,4α = 4; 4α,5β = 11; 7α,8 = 6; 8,9 = 10; 8,11 = 2; 9,11 = 6.5; 11α,12α = 3.5; 17a,17b = 9. Nicotinoyl: 2‴,4‴ = 2; 2‴, 6‴ = 0.8; 4‴,5‴ = 8; 4‴,6‴ = 2.1; 5‴,6‴ = 4.8. Propionyl: 1′,2′ = 7. Butanoyl: 1″,2″ = 2″,3″ = 7.

those reported for derivatives of lathyrol, therefore, the compounds obtained in this work were based on the tetracyclic lathyrane skeleton.

The ^{13}C NMR (APT) spectrum of **1** (Table 2) showed signals for 34 carbon atoms corresponding to six CH_3, five CH_2, 13 CH and 10 quaternary C. The multiplicities were assigned by DEPT experiments. Five signals for carbonyl functions were present. One of them, at δ202.9, suggested a seven-membered ring ketone, whereas the others, at δ173.7, 171.7, 170.1 and 164.4 belonged to the ester carbonyls. These data were in accordance with the structure **1**. The positions of the ester functions were partially assigned by transesterification reactions [8], and by comparison with the results of selective INEPT experiments on **2**, as described below. In the ^{1}H NMR spectra, the proton signals for the diterpene moiety were almost the same in all four compounds; they only differ in the nature and the location of the respective ester groups.

Compounds **2** and **3** showed slightly different R_f values 0.30 and 0.24, respectively, and both compounds afforded a molecular ion peak at *m/z* 637 suggesting the molecular formula, $C_{35}H_{43}NO_{10}$. In their ^{1}H NMR spectra, they had small shift differences for the ester protons appearing between δ2.30 and 0.85. The ^{1}H and ^{13}C NMR spectra, together with the mass spectral data $[M]^+$, 637 of **2** and **3**, indicated that they were tetraester derivatives of a diterpene alcohol, $C_{20}H_{28}O_6$, esterified by propionic, butanoic, acetic and nicotinic acid moieties. These groups were deduced by mass fragmentations with ions at *m/z* 57, 71, 60, 106, respectively. All proton signals were assigned by spin decoupling experiments (Table 3) on **2** in C_6D_6. The ^{13}C NMR (APT) spectrum of **2** displayed 35 carbons. All of the signals were assigned in combination with the ^{1}H–^{1}H and ^{1}H–^{13}C COSY and DEPT experiments.

Table 2. ^{13}C NMR spectal data of compounds **1**–**4** (in $CDCl_3$, 50.32 MHz)

C		1	2	3	4
1		42.2	42.1	42.2	42.2
2		36.6	36.6	36.9	36.7
3		77.2	77.2	77.2	77.2
4		52.9	52.9	52.9	52.9
5		66.7	66.7	66.8	66.7
6		55.5	55.4	55.5	55.5
7		66.2	66.3	66.2	66.3
8		121.9	121.9	121.9	121.9
9		135.1	135.1	135.1	135.1
10		146.3	146.2	146.3	146.3
11		41.8	41.8	41.8	41.3
12		42.9	42.9	42.9	42.9
13		90.6	90.5	90.6	90.5
14		202.9	203.5	203.4	203.5
15		90.1	90.1	90.1	90.1
16		14.3	14.4	14.4	14.4
17		70.0	69.9	69.9	70.0
18		113.5	113.5	113.5	113.5
19		21.1	21.2	21.1	21.1
20		20.8	20.7	20.8	20.8
$COCH_3$		170.1	169.9	169.9	170.0
$COCH_3$		21.1	21.1	21.0	21.1
propionyl	1′	173.7 (171.7)	173.6	173.0	
	2′	27.6	27.8	27.3	
	3′	8.7	8.8	8.7	
butanoyl	1″	—	171.1	171.6	171.1
	2″	—	35.8	36.3	36.3
	3″	—	17.7	17.6	17.7
	4″	—	13.6	13.8	13.8
nicotinoyl	2‴	150.7	150.6	150.7	150.7
	3‴	126.5	126.4	126.5	126.5
	4‴	137.5	137.1	137.1	137.5
	5‴	123.2	123.1	123.1	123.5
	6‴	153.1	153.1	153.0	153.0
	7‴	164.4	164.4	164.5	164.4

Although the ^{1}H NMR spectrum indicated the nature of the ester functions, no definitive information regarding the location of the acylating groups could be obtained. These assignments for **2** were made, in part, by a transesterification reaction with 0.1 M sodium methoxide–methanol at room temperature which furnished **2a**. The ^{1}H NMR spectrum of **2a** showed a −0.61 ppm diamagnetic shift for H-5 at δ5.49 compared with that of the original spectrum of **2**. In addition, the triplet at δ0.85 and the triplet at δ2.28 were missing, and the signal at δ1.45 was simplified, indicating that a butanoic acid residue was removed from C-5 by transesterification. The other protons geminal to ester functions remained unchanged. Compound **2a** was then treated with 0.2 M sodium methoxide–methanol overnight, the striking difference in the product was the absence of an acetoxymethyl signal at δ2.22 in its ^{1}H NMR spectrum, suggesting that the acetyl group was at C-15. These findings were supported by the ^{13}C NMR (APT) spectrum by disappearance of the resonances at δ171.1, 35.8, 17.7, 13.6 for butanoyl and δ169.9, 21.1 for acetyl groups. The precise locations of the acid moieties could not be completely elucidated by a transesterification.

The location of each ester group in **2** was established through a sequence of selective INEPT experiments [9–12] (Table 4). Protons H-3, H-5 and H-7 were irradiated to establish the three-bond enhancement with the carbonyl carbon of each side chain at C-3, C-5 and C-7, respectively. Irradiation of H-7 enhanced the signal at δ164.4 corresponding to the carbonyl carbon of the nicotinoyl group. Irradiation of H-2‴ (δ9.16) and H-4‴ (δ8.24) also enhanced this carbonyl group resonance confirming that the nicotinoyl side chain is attached to C-7. The irradiation of H-3, as well as the irradiation of methyl protons of the propionyl moiety (H-3′), enhanced the signal at δ173.6 establishing that the propanoyloxy group is attached to C-3. Finally, irradiation of H-5 and the methylene protons at δ1.45 (H_2-3″) enhanced the same carbonyl group (C-1″, δ171.1) demonstrating that the butanoyl moiety is located at C-5. Consequently, the acetyl group must be attached to C-15, as supported by the transesterification reaction. Attempts to irradiate the signals of the acetyl proton as well as H_2-2′ and H_2-2″ in order to observe the two-bond enhancements led to ambiguous results due to the proximity of these three signals in the ^{1}H NMR spectrum. Accordingly, **2** was

Table 3. ^{1}H NMR spin-decoupling experiments with compound **2** (in C_6D_6 200 MHz)

Irradiated δ (ppm) H	Observed δ (ppm) H	Multiplicities changes	
5.55 (H-9)	6.15 (H-8)	*ddd—dd*	(J = 2,6 Hz)
	3.17 (H-11)	*br t—d*	(J = 3.5 Hz)
3.17 (H-11)	3.37 (H-12)	*d—s*	
	5.55 (H-9)	*dd—d*	(J = 10 Hz)
3.70 (H_a-1)	1.40 (H_b-1)	*dd—d*	(J = 11 Hz)
2.27 (H-4)	6.29 (H-5)	*d—s*	
	5.20 (H-3)	*t—d*	(J = 4 Hz)
2.10 (H-2)	3.70 (H_a-1)	*dd—d*	(J = 14 Hz)
	1.40 (H_b-1)	*dd—d*	(J = 14 Hz)
	5.20 (H-3)	*t—d*	(J = 4 Hz)
	0.73 (H_3-16)	*d—s*	
3.90 (H_a-17)	3.50 (H_b-17)	*d—s*	
	0.73 (H_3-16)	*d—s*	
1.51 (H_2-3″)	0.79 (H_3-4″)	*tq—s*	
	2.08 (H_2-2″)	*t—br s*	
2.24 (H_2-2′)	1.03 (H_3-3′)	*t—s*	
5.17 (H-7)	6.15 (H-8)	*ddd—dd*	(J = 2,10 Hz)

Table 4. Selective INEPT experiments on compond **2** in $CDCl_3$ at 90.8 MHz*

Proton irridiated H	δ(ppm)	Carbons enhanced
5	6.10	171.1 (C-1″); 77.2 (C-3); 69.9 (C-17); 55.4 (C-6); 52.9 (C-4)
3	5.24	173.6 (C-1′); 90.1 (C-15); 42.1 (C-1)
7	5.17	164.4 (C-7‴); 135.1 (C-9); 121.9 (C-8); 55.4 (C-6); 42.9 (C-12)
3′	1.04	173.6 (C-1′); 27.8 (C-2′)
3″	1.44	171.1 (C-1″); 90.1 (C-15); 36.6 (C-2); 35.8 (C-2′); 14.4 (C-16); 13.6 (C-4‴)
2‴	9.16	164.4 (C-7‴); 137.1 (C-4‴); 126.4 (C-3‴)
4‴	8.24	164.4 (C-7‴); 153.1 (C-6‴); 150.6 (C-2‴)

identified as 15-*O*-acetyl-3-*O*-propinoyl-5-*O*-butanoyl-7-*O*-nicotinoylmyrsinol. In the view of these findings, the only difference between **2** and **3** was the location of the propionyl and butanoyl groups.

In the ^{1}H NMR spectrum of **4**, in addition to the signals of the diterpene moiety, signals for one acetyl, one nicotinoyl and two butanoyl groups were present. All of the spectral data indicated that the butanoyl ester groups are at C-3 and C-5. The CI-mass spectrum, giving the $[M]^+$ at m/z 651, supported the formula $C_{36}H_{45}NO_{10}$.

Compounds **1–4** were tested for HIV-1 RT inhibitory activity, and **3** and **4** showed moderate inhibitory activity with IC_{50} values of 80 and 67 $\mu g\,ml^{-1}$, respectively, while **1** and **2** were inactive ($IC_{50} > 200\ \mu g\,ml^{-1}$) (Table 5).

Table 5. HIV-1 RT % inhibitory activity of fractions and compounds **1–4** from *E. myrsinites*

Fractions	Tannin	% Inhibition at 200 $\mu g\,ml^{-1}$	Activity
6	+ + +	99.6	after removed tannin* % inhibition at 200 $\mu g\,ml^{-1}$ = 48.2% (weakly active)
9	+	99.3	after removed tannin* % inhibition at 200 $\mu g\,ml^{-1}$ = 98.1% (active)
Compounds		% Inhibition at 200 $\mu g\,ml^{-1}$	IC_{50} ($\mu g\,ml^{-1}$), r^2
1		49.5 (weakly active)	—
2		5.46 (inactive)	—
3		93.1	79.9 $\mu g\,ml^{-1}$, r^2 = 0.995 (moderately active)
4		92.7	66.8 $\mu g\,ml^{-1}$, r^2 = 0.990 (moderately active)

*Tannin was removed by using insoluble PVP only when the sample showed a positive result (+) with $FeCl_3$ test.

EXPERIMENTAL

Instruments. ^{1}H NMR, 200 MHz, ^{13}C NMR 50.32 MHz Bruker AC; Nicolet NMC-360 for selective INEPT experiments; UV, Varian Techtron 635; IR, Perkin Elmer 983. MS, VG Zabspec.

Material. Euphorbia myrsinites L. was collected in August, 1991 from Turkey (Bursa). A voucher is deposited in the Herbarium of Faculty of Pharmacy, University of Istanbul (ISTE:63219).

Extraction and isolation. The roots (260 g) and the aerial parts (965 g) were extracted separately with Me_2CO at room temp. for 3 days. The extracts were concd *in vacuo*, and tested against the P-388 cell line (0.73 and 0.88 μg ml^{-1} respectively). As they both showed activity, they were combined. Concd Me_2CO extract (29 g) was dissolved in a small amount of MeOH–H_2O (2: 1) and partitioned against *n*-hexane, further extract of MeOH–H_2O phase with $CHCl_3$ afforded a mixture of diterpenes. From the *n*-hexane extract (11 g), compounds **5–10** were isolated. For sepn procedures see ref. [3]. The $CHCl_3$ extract (13 g) was subjected to CC on silica gel (250 g) using a gradient of hexane–EtOAc. Ten frs of 100 ml were collected and monitored by TLC. Relatively polar frs 6 and 9 showed HIV-1 RT inhibitory activity. These two frs were combined and further purified by repeated prep. TLC using toluene–Me_2CO (9:1; 4:1) as developing solvents and **1–4** were isolated at R_f 0.42, 0.30, 0.24 and 0.36, respectively. The yields were as follows: 25.2 mg (**1**), 36 mg (**2**), 20 mg (**3**) and 27 mg (**4**).

Spectroscopic data. ^{1}H, ^{13}C NMR (Bruker AC) and spin decoupling experiments of **1–4** are given in Tables 1–3. The selective INEPT experiments were recorded with a Nicolet NMC-360. Proton pulse widths were calibrated using HOAc in 10% C_6D_6 (^1J = 6.7 Hz) in a 5-mm NMR tube [13]. The radiofrequency field strength for the soft pulse was of the order of 25 Hz, and 7 Hz was used as $^3J_{CH}$ for aromatic protons and 5 Hz for aliphatic protons for these experiments.

15-O-*Acetyl*-3,5-O-*dipropionyl*-7-O-*nicotinoylmyrsinol* (**1**). IR ν^{CHCl_3} cm^{-1}: 1740 and 1280 (CO_2R), 1720 (C=O), 1660 (C=C), 1640, 1595, 1460, 1380, 1240, 905, 800, 760. UV λ^{MeOH} nm: 262, 224. CI-MS *m/z*: 623 [M]$^+$, 595, 565, 553, 551, 537, 521, 491, 442, 416, 367, 337, 311, 293, 265, 237, 175, 166, 124, 106, 75, 61, 58.

15-O-*Acetyl*-3-O-*propionyl*-5-O-*butanoyl*-7-O-*nicotinoylmyrsinol* (**2**) *and* 15-O-*acetyl*-3-O-*butanoyl*-5-O-*propionyl*-7-*O*-*nicotinoylmyrsinol* (**3**). IR ν^{CHCl_3} cm^{-1}: 1738 and 1277 (CO_2R), 1720 (C=O), 1660 (C=C), 1645, 1597, 1455, 1379, 1240, 960, 910, 760. UV λ^{MeOH} nm: 260, 225. CI-MS *m/z*: 637 [M]$^+$, 609, 567, 549, 535, 521, 494, 461, 405, 387, 355, 338, 296, 175, 166, 124, 106, 71, 60, 58.

15-O-*Acetyl*-3,5-O-*dibutanoyl*-7-O-*nicotinoylmyrsinol* (**4**). IR ν^{CHCl_3} cm^{-1}: 1737 and 1279 (CO_2R), 1718 (C=O), 1660, 1645, 1597, 1463, 1380, 1240, 907, 900, 760. UV λ^{MeOH} nm: 262, 224. CI-MS *m/z*: 651 [M]$^+$, 591, 565, 529, 523, 505, 470, 441, 416, 318, 311, 293, 265, 175, 166, 124, 106, 87, 71, 60.

Transesterification of compound **2**. Compound **2** (9 mg) was treated with 0.1 M NaOMe–MeOH (2 ml) for 5 hr. The reaction mixture was neutralized with Na-Pi buffer to pH 6.8. After evaporating the MeOH, the aq. phase was extracted with EtOAc, whereupon **2a** was obtained. ^{1}H NMR ($CDCl_3$): δ9.13 *d* (H-2‴), 8.75 *dd* (H-6‴), 8.17 *dt* (H-4‴), 7.35 *dd* (H-5‴), 6.03 *ddd* (H-8), 5.78 *dd* (H-9), 5.49 *d* (H-5), 5.16 *t* (H-3), 5.12 *d* (H-7), 5.00 *br s* (H_2-18), 4.06 *d* (H_a-17), 3.73 *d* (H_b-17), 3.27 *d* (H-12), 3.30 *dd* (H-11), 2.82 *dd* (H_a-1), 1.47 *dd* (H_b-1), 1.86 *s* (H-19), 1.39 *s* (H-20), 1.04 *t* (H-3′), 2.19 *q* (H-2′), 2.45 *dd* (H-4), 2.31 *ddd* (H-2). ^{13}C NMR ($CDCl_3$): δ203.0 (C-14), 173.1 (C-1′), 164.6 (C-7‴), 153.0 (C-6‴), 150.6 (C-2‴), 145.3 (C-10), 136.6 (C-4‴), 135.9 (C-9), 126.2 (C-3‴), 123.0 (C-5‴), 120.6 (C-8), 114.1 (C-18), 88.6 (C-13), 79.6 (C-15), 69.4 (C-17), 69.1 (C-5), 69.0 (C-7), 58.4 (C-6), 54.8 (C-4) 50.4 (C-1), 42.1 (C-12), 40.5 (C-11), 35.8 (C-2″), 32.9 (C-2), 27.6 (C-2′), 8.6 (C-3′).

HIV-1 RT assay. Assays were conducted as described previously [14]. The standard assay mix. (final vol., 100 μl) contained; 50 mM Tris–HCl buffer (pH 8), 150 mM KCl, 5 mM $MgCl_2$, 0.5 mM ethylene glyco-bis-(β-aminoethylether) *N,N′*-tetraacetic acid (EGTA), 5 mM dithiothreitol, 0.3 mM glutathione, 2.5 μg ml^{-1} bovine serum albumin, 41 μM poly A {ε_{260} (mM) = 7.8}, 9.5 μM oligo $(dT)_{12-18}$ {ε_{260} (mM) = 5.6}, 20 μM TTP, and 0.5 μCi [^{3}H]TTP. The reaction was initiated by the addition of 10 μl (0.08 μg) of HIV-1 RT, and the mixt. was permitted to incubate at 37° for 1 hr. Reactions were terminated by the addition of 25 μl of 0.1 M EGTA followed by chilling in ice. Aliquots of each reaction mixt. (100 μl) were then spotted uniformly on to circular 2.5 cm DE-81 (Whatman) filters, kept at ambient temp. for 15 min, and washed ×4 with 5% aq. $Na_2HPO_4 \cdot 7H_2O$. This was followed by two more washings with doubly distilled H_2O. Finally, the filters were thoroughly dried and subjected to scintillation counting.

For testing enzyme inhibition, five serial dilutions of samples in DMSO (10 μl) were added to the reaction mixts prior to the addition of enzyme. The median inhibitory concn (IC_{50}) was calculated from a linearly regressed dose–response plot of percent control activity vs. concentration of compound, utilizing at least 5 concns of each compound. Each data plot represents the average of dublicate tests.

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