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OREVACTAENE,[1] A NOVEL BINDING INHIBITOR OF HIV-1 REV PROTEIN TO REV RESPONSE ELEMENT (RRE) FROM *EPICOCCUM NIGRUM* WC47880

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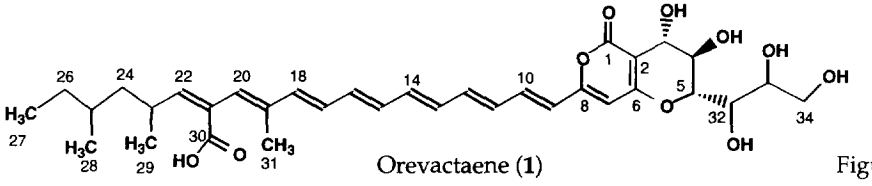
Abstract: Orevactaene (**1**), a novel oxopolyene, was isolated from *Epicoccum nigrum* WC47880 during the screening of microbial fermentation extracts for their ability to inhibit the binding between HIV-1 regulatory protein Rev and its viral RNA-binding site, Rev response element (RRE). The structure of **1** was elucidated by spectroscopic methods. Compound **1** displayed inhibitory activity against the Rev/RRE binding with an IC_{50} value of 3.6 μM. © 1997 Elsevier Science Ltd.

Human immunodeficiency virus type 1 (HIV-1) encodes Rev, a transactivator protein of viral gene expression.[2] Rev accumulates and acts in the nucleoli of expressing cells through the recognition of and specific binding to a highly structured viral mRNA sequence, Rev response element (RRE). Interaction of Rev and RRE regulates the accumulation of viral mRNAs in the cytoplasm of infected cells.[3] Without these mRNAs, structural proteins do not accumulate and the virus cannot replicate.[4] Thus agents blocking this highly specific Rev/RRE interaction have the potential for inhibition of HIV replication.[5]

As a part of our continuing effort for the search of novel inhibitors of Rev/RRE interaction from synthetic and natural sources,[6] a screen was developed and implemented in a high throughput mode. The screen is based on measurement of inhibition of specific binding between Rev protein and RRE[7] as described in the previous publications by these laboratories.[6] As a result, an extract of the fungal solid fermentation culture of *Epicoccum nigrum* WC47880[8] was found to exhibit the inhibitory activity. When subjected to bioassay-guided fractionation, the extract yielded an active compound, orevactaene (**1**). This report describes the isolation, structure determination, and inhibitory effect of HIV-1 Rev/RRE binding of **1**.



Orevactaene (**1**)

Figure 1.

Each step of fractionation was monitored by the Rev/RRE binding assay. Methanol extracts of the solid fermentation culture (20 Nunc plates) of *E. nigrum* WC47880 grown on an agar-based medium[9] were partitioned between *n*-butanol and water. The residue of *n*-butanol layer was chromatographed on HP-20 Diaion resin

(Mitsubishi Kasei America, White Plains, NY) with step solvent gradient elution of water, 30%, 50%, 80% aq MeOH and MeOH. The activity was distributed interestingly in 100% water and 100% MeOH fractions, which upon HPLC analyses in a well-buffered solvent system (pH 3.5) were shown to contain the same major active component (**1**), implying the presence of ionic and free forms of a molecular species in polar and nonpolar fractions, respectively. The active aqueous fraction was thus acidified to pH 3 and extracted with ethyl acetate. The enrichment of activity of ethyl acetate extracts was achieved by medium pressure liquid chromatography (MPLC) on C_{18} with 60% acetonitrile in 0.01 N KH_2PO_4 (pH 3.5) to yield an orange powder (75 mg) with mp 110 °C (dec.) and $[a]_D$ 53.9° (*c* 0.11, DMSO). Compound **1** displayed inhibitory activity against Rev/RRE binding with an IC_{50} value of 3.6 μM.[10] The compound also demonstrated moderate cytotoxicity (IC_{50}: 82 μM) on the murine tumor cell line M109.[11]

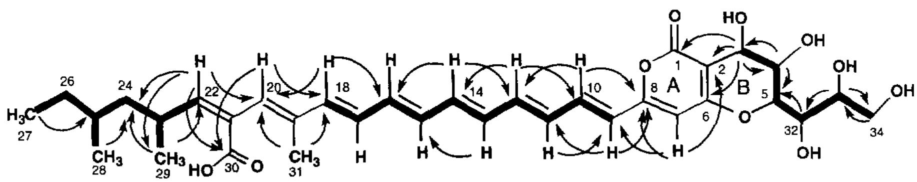


Figure 2. Connectivities of **1** observed in NMR spectra
▬ ^{1}H-^{1}H COSY ⟶ HMBC (H→C)

Compound **1** has the molecular weight of 612 and the molecular formula of $C_{34}H_{44}O_{10}$ from ESMS and HRFABMS (found 613.3044, calcd for MH^+, $C_{34}H_{45}O_{10}$ 613.3012) measurements, indicating an unsaturation degree of thirteen. The major UV maximal absorption at 432 nm suggested the presence of an unusually extended oxopolyene, possibly longer than oxoheptaene.[12] The IR spectrum showed the strong absorptions characteristic of hydroxyls (3415 cm^{-1}), conjugated carbonyls (1635-1684 cm^{-1}), and additional absorptions suggestive of the presence of ester/lactone (1138 cm^{-1}). The ^{1}H-, ^{13}C-, DEPT, and HETCOR NMR spectra (Table 1) in DMSO-d_6 revealed the presence of a total of 34 carbons, including four methyls (a primary methyl, two secondary methyls and a tertiary methyl attached to olefin), two methylenes, two methines, an oxymethylene, five oxymethines, fourteen olefinic methines, two olefinic quaternaries, two quaternary enol carbons (δ_C 157.8 and 162.2) and two non-ketone carbonyls (δ_C 167.6 and 169.6). The broad singlet (δ_H 8.00) due to an exchangeable proton in ^{1}HNMR again suggested the presence of a carboxylic acid group assignable to one of the non-ketone carbonyls. The COSY spectral analysis of **1** enabled the assembly of three partial structural moieties (indicated as bold bonds in Fig. 2); the alkyl moiety C22-C23(C29)-C24-C25(C28)-C26-C27, the polyol moiety C3-C4-C5-C32-C33-C34, and the linear polyene moiety C9 through C18. The long range couplings of H22 *vs* C24/C29/C20/C30 in HMBC spectrum (Fig. 2) allowed the linkage of the alkyl moiety to C21(C30 COOH), and further to C20; strong HMBC correlations of the olefinic methine H-20 singlet *vs* C18/C30 and tertiary methyl H-31 singlet *vs* C20/C18 in turn allowed the extension of C20 to C19(C31 Me) and to C18. Beginning with C18 through C9 the polyene moiety remained unsubstituted, and each of the double bonds was well polarized. The measured coupling constants of H-9, H-10 and H-12 (Table 1) indicated 9(10)*E* and 11(12)*E* geometric configurations. Coupling constants of other ^{1}H-resonances (δ 6.40-6.45) of C18 through C9, though not accurately measurable due to overlapping, all appeared larger than 10 Hz, suggesting all *trans* (*E*) geometric configurations. The long range couplings of H-9 *vs* C8 and H-7 *vs* C9/C8/C2 revealed the connectivity of C9 to the enol double bond C8=C7, followed by another enol

double bond C6=C2. The C3 end of the polyol moiety was linked, based on the key HMBC correlations of H-3 *vs* C1/C2/C6, to C2=C6 double bond and in three bond proximity (possibly *peri* relation) to the C6 carbonyl. Thus far, eleven unsaturation degree (USD) were accounted for by nine double bonds, a carboxylic and an ester/lactone carbonyls. To meet the total USD of thirteen for **1**, a bicyclic hydrogenated pyrano[4,3-*b*]-α-pyrone skeleton was proposed by constructing C1-O-C8 lactone (ring A), and cyclizing C5 oxymethine and C6 enol (ring B), respectively (Fig. 2). This proposed ring system was supported by the large ^{2}J ^{1}H-^{1}H coupling constants (9.3-9.5 Hz) of three carbinol protons (H-3, H-4, and H-5) on ring B, indicating that they are all oriented axially and *trans* to the neighboring proton(s) in a typical six-membered pyran ring system. The ring system was also supported by tandem mass spectrometry (MS/MS) data of the *quasi* molecular ion ($M-H^-$, *m/z* 611) of **1** as shown in Figure 3. Although the relative stereochemistry of ring B was examined, the stereochemistry of other chiral centers are not determined in this study.

Table 1. ^{1}H and ^{13}CNMR Data of Orevactaene (**1**) (DMSO-d6)

No.	δ_C (mult)	δ_H (mult, *J* = Hz)	No.	δ_C (mult)	δ_H (mult, *J* = Hz)
1	167.6 (s)		18	139.1 (d)	6.40 (d, overlapped)
2	101.4 (s)	-	19	134.7 (s)	-
3	74.5 (d)	4.25 (d, 9.5)	20	130.6 (d)	6.11 (s)
4	67.9 (d)	4.11 (dd, 9.5, 9.3)	21	131.4 (s)	-
5	75.3 (d)	3.27 (dd, 9.3, 2.8)	22	146.7 (d)	5.56 (d, 10.3)
6	162.2 (s)	-	23	31.5 (d)	2.90 (m)
7	101.6 (d)	6.18 (s)	24	44.1 (t)	1.08 (m), 1.24 (m)
8	157.8 (s)	-	25	32.2 (d)	1.20 (m)
9	122.5 (d)	6.30 (d, 15.2)	26	29.7 (t)	1.10 (m), 1.20 (m)
10	134.7 (d)	7.00(dd, 15.2, 11.4)	27	11.3 (q)	0.80 (t, 7.6)
11	131.8 (d)	6.45 (dd, overlapped)	28	18.9 (q)	0.78 (d, 7.2)
12	138.7 (d)	6.70 (dd, 14.8, 11.3)	29	21.4 (q)	0.96 (d, 7.1)
13	129.0 (d)	6.42 (dd, overlapped)	30	169.6 (s)	8.00 (br. s, COOH)
14	136.4 (d)	6.51 (dd, overlapped)	31	13.3 (q)	1.79 (s)
15	133.1 (d)	6.41 (dd, overlapped)	32	69.1 (d)	3.70 (dd, 6.2, 2.8)
16	135.5 (d)	6.47 (dd, overlapped)	33	79.3 (d)	3.37 (m, overlapped)
17	132.8 (d)	6.40 (dd, overlapped)	34	60.9 (t)	3.39 (m), 3.42 (m)

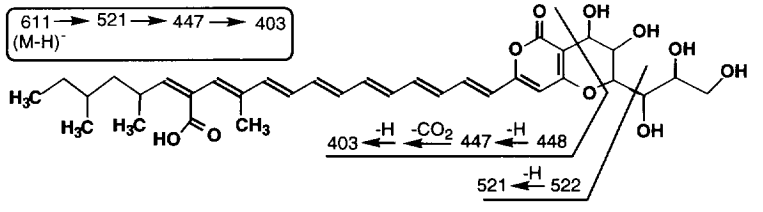


Figure 3. MS/MS product ions of the $M-H^-$ (*m/z* 611) ion of **1** and their substructures

The structure of orevactaene (**1**) represents a novel polyene natural product of a new structural class.[13] The remotely related compounds with similar ring system or polyene portion of **1** are fungal metabolite radicinol[14] or citreomontanine,[15] respectively.

ACKNOWLEDGMENTS

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8. *E. nigrum* WC47880 is a fungus isolated from leaves of *Tiarella cordifolia*.

9. A spore suspension of *E. nigrum* WC47880 was made from a potato dextrose agar (PDA) slant and transferred onto a 24.5 x 24.5 cm Nunc plate containing 250 ml of medium. The culture was incubated at 28 °C for 7 days. The medium contained the following per liter of deionized water: 3 g glucose, 10 g mashed potato, 20 g corn meal, 5 g fish meal, 10 g wheat meal, 10 g cane molasses, 3 g NaCl, 3 g $CaCO_3$, 10 g agar, and the pH was adjusted to 7.0 prior to sterilizing.

10. Compound **1** was also evaluated for protection from HIV-1 infection of CEM-SS cells. Six days after virus infection and co-incubation with a series dilutions of **1** cells were stained with XTT and cell viability was quantified spectrophotometrically. Compound **1** did not show significant anti-HIV activity.

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