Tetrahedron Vol. 49, No. 45, pp. 10435-10438, 1993
Printed in Great Britain

0040-4020/93 $6 00+.00

Petrosynol and Petrosolic Acid, Two Novel Natural Inhibitors of the Reverse Transcriptase of Human Immunodeficiency Virus from Petrosia Sp.

Sara Isaacs, Yoel Kashman*

School of Chemistry, Tel-Aviv University, Ramat Aviv 69978, ISRAEL

Shoshana Loya and Amnon Hizi

School of Medicine, Tel-Aviv University, Ramat Aviv 69978, ISRAEL

Yossi Loya

Department of Zoology, Tel-Aviv University, Ramat Aviv 69978, ISRAEL

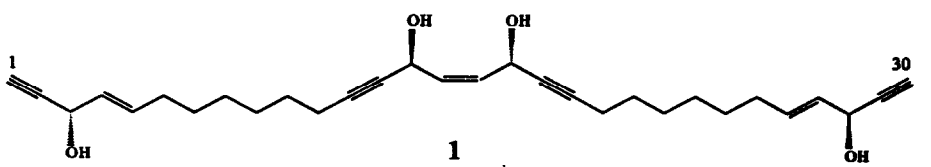
(*Received in UK* 13 *July* 1993; *accepted* 10 *September* 1993)

Abstract: Two polyacetylenes from the Red Sea sponge *Petrosia sp.*, Petrosynol (**1**), and the novel marine natural product petrosolic acid (**2**) were found effective in inhibitory of the DNA polymerase activities of the reverse transcriptase of human immunodeficiency virus. The structure of compound **2** a novel C_{44} oxo-octahydroxy-trienetetraynoic carboxylic acid was determined mainly by NMR spectroscopy.

Reverse transcriptase (RT) is the key enzyme in the life cycle of human immunodeficiency virus (HIV), the etiologic agent of acquired immunodeficiency syndrome (AIDS). RT is a multifunctional enzyme responsible for the transcription of viral RNA into double-stranded DNA. The same protein exhibits both RNA-dependent DNA polymerase (RDDP) and DNA-dependent DNA polymerase (DDDP) activities as well as an inherent ribonuclease H (RNase H) activity. All the catalytic functions of RT play a pivotal role in HIV replication.[1] It is clear, therefore, why this enzyme has been selected as a major target for the specific chemotherapeutic treatment of HIV infection.

In the course of our search for novel natural products active against HIV-RT[2] we have found that the MeOH-CH_2Cl_2 extract of the Red Sea sponge *Petrosia sp* inhibits the RDDP activity of HIV-1 RT. The activity was traced to two polyacetylene compounds **1** and **2** which were isolated upon solvent partition and Sephadex LH-20 chromatographies. Polyacetylenes, are well known from the genus *Petrosia*; several C_{30} to C_{55} polyacetylene alcohols have been isolated and identified during the last decade.[3]

The first polyacetylene, **1**, $C_{30}H_{40}O_4$ (*m/z* 465 MH^+), was found to be identical with petrosynol, the symmetric sea urchin cell division inhibitor, earlier isolated by Fusetani from a *Petrosia* sp.[4]



1

Compound **2**, designated petrosolic acid, was rather labile at room temperature but stable at -15°. The presence of several hydroxyls, disubstituted and terminal acetylenes, and a carbonyl function was inferred from IR(3450 broad and very strong, 3300, 2250, 2100, 1705 cm^{-1}) and the NMR spectra. (Table 1). The NMR data of **2** (125 MHz and 500 MHz, $CDCl_3$ -d_4MeOH 1:4) revealed:

a. eight methinoxy groups (δ_C 49.9d-75.5d; δ_H 4.06-5.08) which were all confirmed by $^2J_{\underline{C}\text{-}O\text{-}\underline{H}}$ correlations observed by a HMBC experiment in d_6-DMSO (δ_{OH} 6.18, 5.62, 5.39, 5.31, 5.23, 4.92, 4.25, 3 .99);the relative high field of six out of the latter eight C-atom resonances suggesting them to be allylic and/or propargylic,

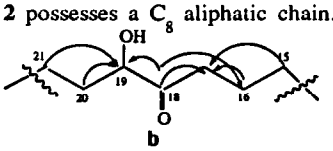
b. seven non protonated sp-carbons (δ_C 79.3s-83.5s) and one terminal methyne (δ_C 72.4d; δ_H 2.82) proposing four triple bonds,

c. six sp^2 C-atoms (δ_C128.2d-133.6d; δ_H 5.28-5.82) suggesting three disubstituted double bonds, and

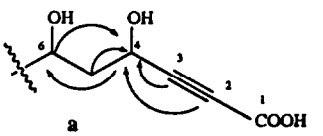
d. twenty methylenes out of which eight overlap (δ_C 28.3; δ_H 1.20).

All the above groups (20 CH_2, 8 CHOH, 3C ≡ C, C ≡ CH, 3-CH=CH, CO) account for $C_{43}H_{63}O_9$. Repeating the ^{13}C NMR spectrum in d_6-DMSO with a delay of 8 seconds revealed an additional signal at 158.6 suggesting a propargyl carboxylic group[5] (δ_C of HC ≡ C - $\underline{C}O_2H$ is 158.3) and hence a molecular formula of $C_{44}H_{64}O_{11}$ (MWt 768). DCI mass spectrum of **2**, *m/z* 751, MH^+-H_2O, was in full agreement with the latter suggested molecular formula. The structure of **2** was determined mainly on the basis of a comprehensive 1D and 2D NMR study. Among d_4-MeOH, d_5-pyridine, d_6-DMSO and d_4-MeOH-$CDCl_3$ 4:1, the last mixture was found to give the best results although for some regions better resolutions were obtained with the other solvents. Table 1 summarizes the NMR data and signal assignments.

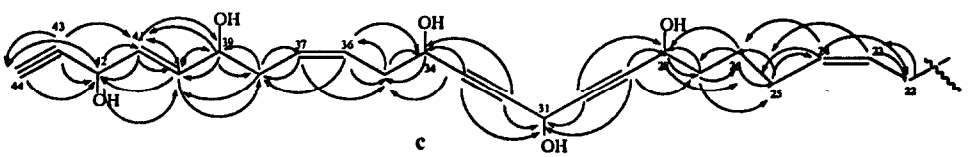
From the HMBC experiments partial structures (**a-c**) could have been suggested, in addition compound **2** possesses a C_8 aliphatic chain.



b

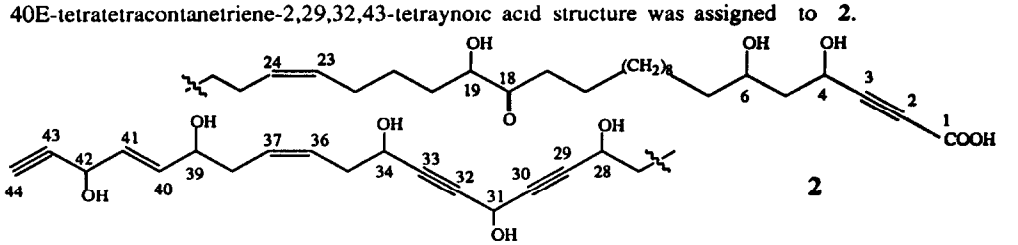


a



c

The protonated moieties were further confirmed by a COSY experiment (e.g. the correlation between H-6 and H_2-7). A TOCSY experiment (correlation between H-21 and H-23) linked **b** to **c**, thus forming a C_{35} block. As the carboxylic acid has to be the second end of the molecule of **2**, **b** has to be linked to **a** through the eight member aliphatic chain. Thus, a 4,6,19,28,31,34,39,42-octahydroxy-18-oxo-23Z, 36Z, 40E-tetratetracontanetriene-2,29,32,43-tetraynoic acid structure was assigned to **2**.



2

The high instability of **2** prevented derivatization of the alcohol groups for absolute configuration assignments.

Compounds **1** and **2** were evaluated for their inhibitory potency against the various activities of HIV-1 RT, i.e., RDDP, DDDP and RNase H functions. In general, compounds **1** and **2** exhibited inhibitory activity of the DNA polymerase functions of HIV-1 RT but almost showed no inhibition of the RT-associated RNase H. Compound **2** was found to be the most potent inhibitor of the RDDP activity (with 50% inhibition obtained at 1.2 μM and 95% at 5.9 μM), wheras the DDDP activity was significantly less sensitive (see Table 2).

Table 1: NMR Data of Compound 2 ($CDCl_3$-d_4MeOH, 1:4, 125 and 500 MHz)

No.	δ_C	δ_H	COSY/ TOCSY	HMBC (H to C)	No	δ_C	δ_H	COSY/ TOCSY	HMBC (H to C)
1	158.6s	—			27	35.9t	1.58,m(2H)	28	29,28,26
2	79.3s	—			28	60.1d	4.24,dt	31	30,29,27,26
3	80.9s	—			29	83.1s	—		
4	57.1d	4.50,dd	5a,5b,6	6,5,3,2	30	80.6s	—		
5	43.4t	1.61,m	5b,6		31	49.9d	5.08,t	34	33,32,30,29
		1.73,m	6						
6	65.9d	3.72,m	7		32	83.5s	—		
7	36.3t	1.35(2H)			33	80.5s	—		
8-15	28.3t	1.20(16H)	17		34	60.1d	4.26,dt	35,36	36,35,33,32
16	27.8t	1.58,m(2H)	17	18	35	34.4t	2.32,m(2H)	36	37,36,34,33
17	36.3t	2.46,m(2H)		18,16,8-15	36	126.8d	[c]5.50,m		38,35
18	213.0s	—			37	125.1d	[c]5.50,m	38,39	38,35
19	75.5d	3.97,dd	20,21,22	21,20,18	38	33.9t	2.27,m(2H)	39	40,39,37,36
20	32.1t	1.52			39	69.8d	4.06,brq	40,41	41,40,38
21	23.1t	1.72	23		40	133.6d	5.82ddd	41,42	42,41,39,38
22	25.6t	[b]1.98,m(2H)	23	24,23	41	128.6d	5.68,ddd	42	43,42,40,39
23	128.2d	[a]5.28,m		25,22	42	60.1d	4.74,brd	44	44,43,41,40
24	128.2d	[a]5.28,m	25,26,27	25,22	43	82.0s	—		
25	25.6t	[b]1.98,m(2H)	28	27,26,24,23	44	72.4d	2.82,d		42,43
26	24.4t	1.40,m(2H)		28,27,25,24					

J values (H to H in Hz): 4,5a=3.5; 4,5b=9.1; 19,20a=4.1; 19,20b=8.0; 27a,28=5.1; 28,31=1.5; 31,34=1.5; 34,35a=5.3; 34,35b=5.3; 38a,39=5.8; 38b,39=5.8; 39,40=5.8; 39,41=1.1; 40,41=15.3; 40,42=1.1; 41,42=5.7; 42,44=2.2. a,b,c. Overlapping signals.

Table 2. Inhibition of HIV-1 reverse transcriptase associated RDDP,DDDP & RNase H functions by 1&2[a]

	RDDP		DDDP		RNase H	
	IC_{50}	IC_{95}	IC_{50}	IC_{95}	IC_{50}	IC_{95}
1	15.8 ± 0.8	38.5 ± 0.5	36.0 ± 2.0	74.0 ± 3.0	>200	>>200
2	1.2 ± 0.3	5.9 ± 1.5	6.2 ± 0.2	16.5 ± 1.7	39.5 ± 4.5	>>200

a. The inhibitor concentration leading to 50% (IC_{50}) and 95% (IC_{95}) inhibition of the initial enzymatic activities of HIV-1 RT, expressed in μM. All data represent mean values (± range) for at least two separate experiments.

EXPERIMENTAL

General Experimental Procedures - IR spectra were recorded on a Nicolet 205 FT-IR spectrophotometer. ^1H and ^{13}C NMR spectra were recorded on Bruker AMX-360 and ARX-500 spectrometers.

All chemical shifts are reported with respect to TMS ($\delta = 0$). LRMS were taken on a Finnigan TSQ-70 instrument and on a Finnigan 4021 instrument. Optical rotations were measured on a Perkin-Elmer Model 141 polarimeter using a 1 dm microcell.

The dry sponge (20g) was extracted (x3) with MeOH-CH_2Cl_2, 1:4, to give a brown gum (ca. 100mg). The latter material was taken into 10% H_2O-MeOH and then partitioned against CCl_4, $CHCl_3$ and after removing the MeOH against n-BuOH. The $CHCl_3$ partitioned phase was chromatographed on Sephadex LH-20, prepared and eluted with $CHCl_3$-MeOH, 1:1, to give compound **1** (10 mg), identical in all respects with petrosynol[4]. Sephadex LH-20 chromatography ($CHCl_3$-MeOH, 1:1) of the residue from the n-butanol partitioned phase afforded compound **2** (40 mg); amorphous powder, $[\alpha]_D^{22} + 7^o$ (C 2.9, MeOH), IR(neat) 3450, 3300, 2250, 2100, 1705 cm^{-1}; found: C, 68.90; H, 8.65; $C_{44}H_{64}O_{11}$ requires: C 68.71; H 8.39%; DCIMS *m/z* (%) 751.6 $[M\text{-}H_2O]^+$ (46), 733.6 $[M\text{-}2H_2O]^+$ (46), 639.5(30), 621.6(31), 475.4 (54), 413.3 (100), for ^1H and ^{13}C-NMR data , see Table 1.

Enzymes: The HIV-1 reverse transcriptases used in this study was a recombinant enzyme, derived from BH-10 proviral clone of HIV-1 and expressed in *Escherichia coli*.[6] The enzyme was purified to near homogeneity as p66/ p51 heterodimer according to the protocol described by Clark et al.[7]

Enzymatic assays: The HIV-1 reverse transcriptase associated RDDP, DDDP and RNase H activities were assayed as described in detail previously.[2,8,9] In short, the RDDP activity was assayed by monitoring the poly(rA)$_n$·oligo(dT)$_{12\text{-}18}$-directed incorporation of [^{3}H]dTTP into trichloroacetic acid-insoluble DNA product. The DDDP activity was asayed with an activated DNA as primer-template and with all four deoxynucleotides present (of which only one, dTTP, was radioactively labeled). The RNase H activity was assayed by measuring the releases of trichloroacetic acid-soluble material from the synthetic substrate [^{3}H]poly(rA)$_n$ poly(dT)$_n$.

Acknowledgements: The research was supported by a grant (AI 31790) from the National Institute of Allergy and Infections Diseases. We thank M. Litvak for technical assistance and Dr. M. Ilan for the identification of the sponge, and Prof. A. Mandelbaum, the Mass Spectroscopy Center, Technion, Haifa.

REFERENCES AND NOTES

1. a. De Clercq, E.; AIDS Res. Human Retrovir. **8**, 119 (1992). b. Coffin, J.M.; In: Virology Ed. by Fields, B.N. and Knipe, D.M. 2nd edition. Raven Press, Ltd. New-York 1990, pp 1437-1500, c. Goff, S.P.; J. AIDS **3**, 817 (1990).
2. Loya, S.; Hizi, A.; FEBS Lett. **269**, 131 (1990); Loya, S.; Tal, R.; Kashman, Y.; Hizi, A.; Antimic. Agents Chemoth., **34**, 2009 (1990); Loya, S.; Kashman, Y.; Hizi, A.; Arch of Biochem. Biophys., **293**, 208 (1992).
3. a. Cimino, G.; De Giulio, A.; De Rosa, S...; Di Marzo, V.; Tetrahedron Lett., **30**, 3563 (1989). b. Cimino, G.; De Giulio, A.; De Rosa, S.; De Stefano, S.; Sodano, G.; J. Nat. Prod. **48**, 22 (1985). c. Castiello, D.; Cimino, G.; De Rosa, S.; De Stefano, S.; Sodano, G.; Tetrahedron Lett.,**21**, 5047 (1980). d. Cimino, G.; Crispino, S.; De Rosa, S.; De Stefano, S.; Sodano, G.; Experimentia, **37**, 924 (1981). e. Fusetani, N.; Li, Hong-yu; Tamura, K.; Matsunaga, S.; Tetrahedron, **49**, 1203 (1993).
4.a. Fusetani, N.; Kato, Y.; Matsunaga, S.; Hashimoto, K.; Tetrahedron Lett., **24**, 2771 (1983).
b. Fusetani, N.; Shiragaki, T.; Matsunaga, S.; Hashimoto, K.; Tetrahedron Lett., **28**, 4313 (1987).
c. Fusetani, N.; New J. Chem., **14**, 721 (1990).
5. The carboxyl proton could have been observed in the d_6-DMSO NMR spectrum at δ_H 13.5 ppm; Micro-methylation of **2** with CH_2N_2 gave the expected methylation of the carboxylic group but also methylation of the 14-OH (δ_H 3.95 and 4.18) TLC hexane-ethylacetate, (1:1) gave Rf=0.4
6. Hizi, A.; McGill, C.; and Hughes, S.H. (1988). Proc. Natl. Acad. Sci. USA **85**, 1218.
7. Clark, P.K.; Ferris, A.L.; Miller, D.A.; Hizi, A.; Kim, K.-W.; Deringer-Boyer, S.M.; Mellini, M.L.; Clark, Jr., A.D.; Arnold, G.F.; Lebherz, W.G.; Arnold, E.; Muschik, G.M. and Hughes, S.H. AIDS Res. Human Retrovir. **6**, 753 (1990).
8. Hizi, A.; Tal, R.; Shaharabany, M.; and Loya, S. J. Biol. Chem. **266**, 6230 (1990).
9. Loya, S.; Tal, R.; Hughes, S.H.; and Hizi, A. J. Biol. Chem. **267**, 13879 (1992).