Pergamon

Bioorganic & Medicinal Chemistry Letters, Vol. 5, No. 6, pp. 605-610, 1995

0960-894X/95 $9.50+0.00

0960-894X(95)00081-X

WIEDENDIOL-A AND -B, CHOLESTERYL ESTER TRANSFER PROTEIN INHIBITORS FROM THE MARINE SPONGE *XESTOSPONGIA WIEDENMAYERI*

Stephen J. Coval,* Michael A. Conover, Ronald Mierzwa, Arthur King, Mohindar S. Puar, David W. Phife, Jin-Keon Pai, Robert E. Burrier, Ho-Sam Ahn, George C. Boykow, Mahesh Patel and Shirley A. Pomponi#

Schering Plough Research Institute, 2015 Galloping Hill Road
Kenilworth, New Jersey 07033 and #Harbor Branch Oceanographic Institute,
5600 US 1 North, Fort Pierce, Florida 34946[1]

Abstract: Wiedendiol-A and -B, novel sesquiterpene-hydroquinones which inhibit cholesteryl ester transfer protein (CETP), have been isolated from the marine sponge *Xestospongia wiedenmayeri*. Additional related marine natural products were also evaluated for CETP inhibition. Compounds that inhibit the activity of CETP may find utility as antiatherosclerosis therapy.

Despite intensive research efforts over the past several decades, cardiovascular diseases remain the major cause of morbidity and mortality in the United States and other industrialized nations.[2] Among the risk factors which have been identified are several plasma lipid related factors, including high levels of low density lipoprotein (LDL), and low levels of high density lipoprotein (HDL).[3] The metabolism of these two lipoproteins is complex and related to one another in several species by the action of cholesteryl ester transfer protein (CETP).[4] In humans, monkeys, hamsters, rabbits and other species CETP transfers cholesteryl esters from HDL to LDL (as well as to LDL precursor lipoproteins VLDL and IDL), with the reciprocal transfer of triglycerides into the HDL fraction. Thus the action of CETP produces an atherogenic lipoprotein profile.

This concept has been verified by identification of patients lacking CETP activity who have unusually high HDL with low LDL.[4] Conversely, transgenic mice expressing high levels of CETP have shown a greater proportion of the plasma cholesterol to be in the VLDL and LDL fractions,[4,5] with some of the animals also showing increased atherosclerosis.[5] These studies suggest that inhibition of CETP will have a direct impact on plasma lipoproteins leading to the reduction of several important risk factors. CETP has been purified, cloned, and the cDNA expressed in bacteria and CHO cells making possible the development of an assay for CETP inhibition.

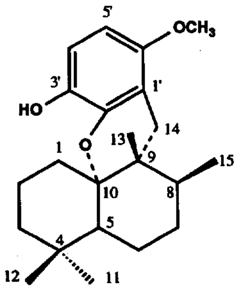
We have discovered, via screening using an Amersham Scintillation Proximity Assay (SPA) kit for CETP inhibition, a family of marine sponge metabolites that inhibit CETP. In this CETP-SPA assay, the transfer of [^{3}H]cholesteryl esters from HDL to biotinylated LDL is measured following incubation of donor ([^{3}H]CE-HDL) and acceptor (biotinylated LDL) particles in the presence of human recombinant CETP. Following incubation at 37°C in 96-well plates (*Dynatech*) for 4 hours, the reaction is terminated by adding

200 ml of streptavidin SPA beads formulated in an assay termination buffer. Transfer is measured after counting cpm on a TopCount 96-well scintillation counter (*Packard*). The rate of increase in signal is proportional to the transfer of $[^3H]$cholesteryl esters by CETP. Initially we isolated three CETP inhibitors from a marine sponge, but subsequently obtained additional related natural products for CETP inhibition evaluation. The inhibition of CETP displayed by these compounds ranged from no inhibition to an IC_{50} of 0.3μM. This is the first report of natural products which display this potentially beneficial activity.

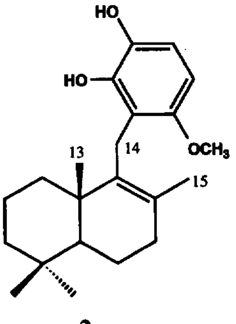
The sponge, *Xestospongia* cf. *wiedenmayeri* van Soest, 1980 (Phylum Porifera, Class Demospongiae, Order Haplosclerida, Family Petrosiidae) was collected by scuba diving at a depth of 37 meters from the fore reef escarpment off northwest Crooked Island, Bahamas. A sample of the sponge was preserved in ethanol as a taxonomic voucher; the remainder of the sponge was stored frozen at -20°C. The voucher specimen is currently deposited at the Harbor Branch Oceanographic Museum, catalog number 003:00073.

The sample described above differs from the published description of *X. wiedenmayeri*[6] in the possession of two categories of strongyles, with occasional tylote modifications, instead of the thick, oxeote spicules reported for *X. wiedenmayeri*. Another difference is in the occurrence of this sponge in a deep reef environment; the specimen described by van Soest was reported to be taken from mangrove roots and muddy environments.

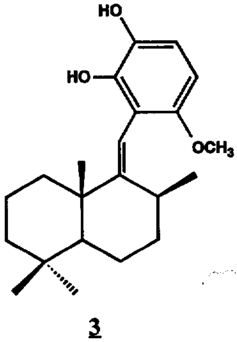
A portion of the sponge was solvent extracted, and the extract subjected to bioassay guided fractionation to give CETP-active compounds **1** (SCH 50678), **2** (SCH 50679) and **3** (SCH 50680). The first compound isolated (**1**) was also the most abundant and was examined first. The molecular formula was determined by high resolution chemical ionization (CI) mass spectral peak matching. The observed $(M + H)^+$ peak at m/z 345.2413 versus the calculated mass of 345.2430 for $C_{22}H_{33}O_3$ suggested the molecular formula $C_{22}H_{32}O_3$ for **1**. Examination of the ^1H and ^{13}C NMR spectra of **1** revealed the presence of a methoxy group and a tetra-substituted benzene ring. The remaining fifteen carbons, with the exception of a singlet at δ 84.1 (C-10), all resonated at high field in the ^{13}C NMR spectrum. These data, in conjunction with the taxonomic source, suggested the possibility of an zonarol[7] type structure. Upon analysis by COSY, HETCOR, SINEPT, and NOESY it was determined that **1** was identical to the antiviral sesquiterpene hydroquinone strongylin A.[8]



1



2



3

Table 1. ^{13}C NMR Assignments (δ)

C#	**1**	**2**	**3**
1	29.3 t	35.9 t	38.8 t
2	18.7 t	18.8 t	18.8 t
3	33.7 t	41.6 t	42.0 t
4	33.5 s	33.5 s	34.0 s
5	44.0 d	51.7 d	55.1 d
6	22.5 t	18.8 t	17.8 t
7	27.8 t	33.6 t	34.2 t
8	39.5 d	133.1 s	32.0 d
9	38.1 s	143.7 s	164.3 s
10	84.1 s	39.6 s	41.3 s
11	32.1 q	33.3 q	33.4 q
12	29.1 q	21.8 q	22.8 q
13	20.1 q	20.0 q	21.9 q
14	32.2 t	24.8 t	112.6 d
15	17.4 q	20.6 q	21.8 q
1'	110.5 s	113.6 s	114.8 s
2'	139.5 s	140.3 s	139.7 s
3'	138.6 s	139.1 s	137.8 s
4'	110.6 d	110.8 d	109.7 d
5'	100.3 d	101.5 d	102.7 d
6'	150.9 s	150.7 s	151.0 s
OMe	55.5 q	55.9 q	56.0 q

Table 2. ^{1}H NMR Assignments[a] (δ)

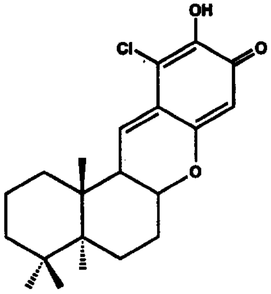
H#	**1**	**2**	**3**
1	1.8	na[b]	na
2	1.9, 1.5	na	na
3	1.4, 1.2	na	na
5	1.5	na	na
6	1.7, 1.6	na	na
7	2.1, 1.4	2.16 2H dd 8.9, 4.3	na
8	1.7	—	2.60 1H m
11	0.75 3H s	0.92 3H s	0.91 3H s
12	1.02 3H s	1.03 3H s	0.91 3H s
13	0.88 3H s	0.86 3H s	1.25 3H s
14a	3.12 1H d 17.5	3.50 1H d 17.2	5.78 1H s
14b	2.11 1H d 17.5	3.42 1H d 17.2	—
15	1.12 3H d 7.4	1.72 3H s	1.03 3H d 7.6
4'	6.68 1H d 8.7	6.69 1H d 8.8	6.76 1H d 8.8
5'	6.26 1H d 8.7	6.30 1H d 8.8	6.36 1H d 8.8
OMe	3.77 3H s	3.77 3H s	3.70 3H s
OH	5.01 1H s	7.48 1H s	5.13 1H s
OH	—	5.14 1H br s	4.97 1H s

[a] Assignments based on HETCOR and SINEPT, [b] Not assigned

The exhaustive spectral analysis of **1** allowed full proton and carbon assignments (Tables 1 and 2), which when used for comparison provided a strong basis for the structure elucidation of compounds **2** and **3**. NMR and high resolution mass spectral analysis of compound **2**, which we give the trivial name wiedendiol-A, indicated it too had the molecular formula $C_{22}H_{32}O_3$. Readily notable in the ^{13}C NMR spectrum of **2** were two olefinic carbon singlets (δ 143.7 s and 133.1 s) that were not present in the spectrum of **1**. Thus compound **2** would necessarily possess one less ring than **1**. The lack of an oxygen bearing sp^3 carbon, and the presence of an additional exchangeable proton, confirmed the likely opening of the ether linkage with the residual hydroxyl located on the aromatic ring. The location of the olefinic bond was determined by comparison of the ^{1}H NMR spectra of compounds **1** and **2**. Missing from the spectrum of **2** was the C-15 methyl doublet (δ 1.12, 3H d in **1**), which was replaced by a vinyl methyl singlet (δ 1.72 3H s in **2**). Additionally, CH_2-14 is shifted downfield in **2** relative to **1** (δ 3.46 in **2** versus 3.12 and 2.11 in **1**). These two differences, and the lack of any other significant changes, imply that the double bond in compound **2** must be between carbons 8 and 9. The substitution pattern on the aromatic ring is the same as in **1**, with the former ether linkage replaced by a phenolic hydroxyl.

Wiedendiol-B (**3**) was found by high resolution mass spectrometry and NMR analysis to possess the same molecular formula as **1** and **2**. Like compound **2**, the ^{13}C NMR spectrum of **3** also shows two olefinic carbons. But unlike **2**, only one of the two olefinic carbon signals is a singlet (δ 164.3 s), while the other is a doublet (δ 112.6 d). The proton NMR spectrum of **3**, like that of compound **1**, shows CH_3-15 as a highfield doublet (δ 1.03 3H d). However, the COSY spectrum of **3** shows that CH_3-15 is coupled to the allylic methine proton H-8 (δ 2.60 m). The lone olefinic proton resonates as a singlet at δ 5.78, but does show long range carbon-proton correlations to C-10, C-8, C-6' and C-1' (SINEPT). Missing from the proton NMR spectrum of **3** is the isolated methylene (CH_2-14) that was present in both **1** and **2**. These data indicate the olefin in **3** is located between C-9 and C-14. The orientation of the double bond was determined to be *Z* by an nOe enhancement observed between H-14 and the methylene protons on CH_2-1. Proton and ^{13}C NMR data for the aromatic ring of **3** is virtually identical to that of compound **2**.

Compounds **1**-**3** are members of a large group of mainly marine secondary metabolites composed of a sesquiterpene linked to an benzenoid ring. As already noted, compound **1** was previously reported, but compounds **2** and **3** are both novel metabolites. Numerous biological activities have been attributed to this class of compounds which include antiviral,[8,9] cytotoxicity,[10] antimicrobial,[11] and inhibition of tubulin polymerization.[12] For a comprehensive survey of the marine derived compounds in this family see the review series by D. John Faulkner.[13] Terrestrial fungi have also been reported to produce a similar set of compounds that differ from the marine metabolites by having additional carbon substitution in the aromatic ring, predominantly at the 4' position. Most of these fungal metabolites display antifungal activity, and are typified by the macrophorins,[14] siccannin,[15] podosporin[16] and stachybotrydial.[17]



4

Table 3. CETP-SPA μM IC_{50}'s

Compound	IC_{50} μM
1 SCH-50678	46
2 SCH-50679	5
3 SCH-50680	5
Puupehenone triacetate[18]	23
Puupehenone[18]	6
Chloropuupehenone[18]	0.3
Avarol[19]	25
Aureol[20]	22
Illimaquinone[21]	100
Spongiatriol[22]	103
Spongiadiol[21]	196
Sulfiricin[23]	>190

The IC_{50} values in the CETP-SPA assay for compounds **1**-**3**, and some marine compounds bearing similar structural features, are shown in Table 3. Compounds with IC_{50} values greater than 30μM are essentially inactive. Of the three described in this report, only **2** and **3** are effective CETP inhibitors. Chloropuupehenone (**4**)[18] is the most active compound tested with an IC_{50} of 0.3 μM. Additional verification of the CETP inhibition of compounds **2** and **3** was carried out using a precipitation method to separate lipoproteins after incubation of radiolabeled HDL with LDL and CETP.[24] In this assay compounds **2** and **3** had IC_{50}'s of 1 and 0.6 μM respectively.

ACKNOWLEDGMENTS

We kindly thank Dr. Alan Tall for recombinant CETP, and Dr. Paul J. Scheuer for supplying samples of avarol, puupehenone and chloropuupehenone.

EXPERIMENTAL

GENERAL: The silica used was EM Science Silica gel 60, 230-400 mesh. NMR spectra were recorded in $CDCl_3$ on a Varian XL-400 spectrometer. The low resolution mass spectra were recorded on a Finnigan MAT-312 mass spectrometer. The high resolution mass spectra were recorded on a VG-ZAB-SE double focusing reverse geometry mass spectrometer.

ISOLATION OF **1**-**3**: A sample (26 g) of the sponge, which had been kept frozen since collection, was lyophilized to give 7.2 g dry sponge. The dry sponge was pulverized to a dry powder and subjected to soxhlet extraction with petroleum ether for 24h. The extract was concentrated under vacuum to give 492 mg of residue. A portion of the residue (471 mg) was chromatographed over silica gel employing gradient elution of heptane through 1:4 heptane/chloroform. This gave 123 mg of **1** (SCH 50678), 14 mg of **2** (SCH 50679), 19 mg of mixed **1** and **2**, and 110 mg of impure **3**. The mixed **1** and **2** was rechromatographed using the same conditions as above to give an additional 8 mg of **1** (total yield of **1** equals 1.9% of dry sponge) and 11 mg of **2** (total yield of 2 equals 0.4% of dry sponge). The fraction containing impure **3** was further fractionated by silica gel chromatography employing a step gradient starting with 1:1 heptane/chloroform and finishing with 9:1 chloroform/methanol to give 25 mg of **3** (SCH 50680, yield 0.4% of dry sponge).

Strongylin A **1** (SCH 50678): $[\alpha]_D^{21.5}$ (chloroform) +85°, UV (heptane) λ_{max} (ε) 201 (52200), 225 sh (9470), 284 (3440), IR (film) cm^{-1} 3560, 3464 br, 2930, 1615, 1491, 1385, 1265, 1185, 1090, 757, HR FAB MS $(M+H)^+$ *m/z* 345.2413, CIMS *m/z* (rel. Int.) 345 (35), 344 (15), 191 (63), 153 (100), ^{1}H and ^{13}C nmr see Tables 1 and 2.

Wiedendiol-A **2** (SCH 50679): $[\alpha]_D^{21.5}$ (chloroform) +121.°, UV (heptane) λ_{max} (ε) 201 (17700), 288 (640), IR (film) cm^{-1} 3498 br, 3340, 2945, 1615, 1492, 1288, 1385, 1258, 1181, 1085, 789, 728, HR FAB MS $(M+H)^+$ *m/z* 345.2410, CIMS *m/z* (rel. Int.) 345 (39), 344 (15), 191 (38), 153 (100), ^{1}H and ^{13}C nmr see Tables 1 and 2.

Wiedendiol-B **3** (SCH 50680): $[\alpha]_D^{21.5}$ (chloroform) -41°, UV (heptane) λ_{max} (ε) 199 (41400), 288 (16100), 325 (9400), IR (film) cm^{-1} 3500 br, 2934, 1596, 1498, 1466, 1377, 1316, 1258, 1208, 1155, 758, HR FAB MS $(M+H)^+$ *m/z* 345.2413, CIMS *m/z* (rel. Int.) 345 (38), 344 (58), 191 (53), 153 (100), ^{1}H and ^{13}C nmr see Tables 1 and 2.

References and Notes

---

1. Harbor Branch Oceanographic Institute Contribution No. 1067.
2. Robinson, J.G.; Leon, A.S. *Med. Clin. North Am.* **1994**, *78*, 69.
3. The Expert Panel: Report of the National Cholesterol Education Program expert panel on detection, evaluation, and treatment of high blood cholesterol levels in adults. *Arch. Intern. Med.* **1988**, *148*, 36.

4. Tall, A.R. *J. Lipid Res.* **1994**, *34*, 1255.
5. Marotti, K.R.; Castle, C.K.; Boyle, T.P.; Lin, A.H.; Murray, R.W.; Melchior, G.W. *Nature* **1993**, *364*, 73.
6. Soest, R.W.M. van *Stud. Fauna Curacao Caribb.* **1980**, *62*, 1.
7. Fenical, W.; Sims, J.J.; Squatrito, D.; Wing, R.M.; Radlick, P. *J. Org. Chem.* **1973**, *38*, 2383.
8. Wright, A.E.; Rueth, S.A.; Cross, S. *J. Nat. Prod.* **1991**, *54*, 1108.
9. Hamann, M.T.; Scheuer, P.J. *Tetrahedron Lett.* **1991**, *32*, 5671.
10. Hirsch, S.; Rudi, A.; Kashman, Y.; Loya, Y. *J. Nat. Prod.* **1991**, *54*, 92.
11. Sullivan, B.W.; Faulkner, D.J.; Matsumoto, G.K.; Cun-heng, H.; Clardy, J. *J. Org. Chem.* **1986,** *51*, 4568.
12. Knodracki, M.L.; Davoust, D.; Guyot, M. *J. Chem. Research* **1989**, 74.
13. Faulkner, D.J. *Nat. Prod. Rep.* **1993**, *10*, 497.
14. Sassa, T.; Yoshikoshi, H. *Agric. Biol. Chem.* **1983**, *47*, 187.
15. Ishbashi, K. *J. Antibiotics Ser A* **1962**, *15*, 161. Hirai, K.; Nozoe, S.; Tsuda, K.; Iitaka, Y.; Ishibashi, K.; Shirasaka, M. *Tetrahedron Lett.* **1967**, 2177.
16. Weber, H.A.; Baenziger, N.C.; Gloer, J.B. *J. Org. Chem.* **1988**, *53*, 4567.
17. Ayer, W.A.; Miao, S. *Can. J. Chem.* **1993**, *71*, 487.
18. Ravi, B.N.; Perzanowski, H.P.; Ross, R.A.; Erdman, T.R.; Scheuer, P.J.; Finer, J.; Clardy, J. *Pure Appl. Chem.* **1979**, *51*, 1893.
19 Minale, L.; Riccio, R.; Sodano, G. *Tetrahedron Lett.* **1974,** 3401.
20. Djura, P.; Stierle, D.P.; Sullivan, B.; Faulkner, D.J.; Arnold, E.; Clardy, J. *J. Org. Chem.* **1980**, *45*, 1435.
21. Luibrand, R.T.; Erdman, T.R.; Vollmer, J.J.; Scheuer, P.J.; Finer, J.; Clardy, J. *Tetrahedron* **1979**, *35*, 609.
22. Kazlauskas, R.; Murphy, P.T.; Wells, R.J.; Noack, K.; Oberhansli, W.E.; Schonholzer, P. *Aust. J. Chem.*, **1979**, *32*, 867.
23. Wright, A.E.; McCarthy, P.J.; Shulte, G.K. *J. Org. Chem.* **1989**, *54*, 3472.
24. Morton, R.E.; Zilversmit, D.B. *J. Biol. Chem.* **1981**, *256*, 11992.

(*Received in USA* 19 *December* 1994; *accepted* 6 *February* 1995)