NOTES

Inhibition of Cholesteryl Ester Transfer Protein by Rosenonolactone Derivatives

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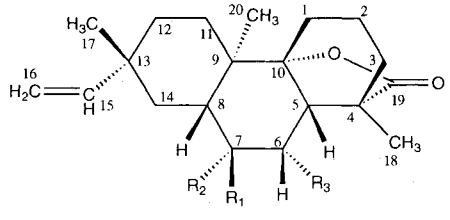
Several recent clinical trials have demonstrated that lower plasma cholesterol levels do indeed have a beneficial effect on events related to coronary heart diseases. Among the risk factors that have been identified are several plasma lipid related factors, including high levels of low density lipoprotein (LDL) and low levels of high density lipoprotein (HDL). Human plasma cholesteryl ester transfer protein (CETP) promotes the exchange of cholesteryl esters and triglycerides between HDL and LDL.[1] CETP has been recognized as one of the important factors determining the atherogenicity of the lipoprotein profile in human plasma.[2] However, it has also been reported that CETP has a potent antiatherogenic function *via* reverse cholesterol transport.[3] CETP may be regarded as a potentially atherogenic factor which bypasses the reverse cholesterol transport pathway by transferring cholesterol esters from the antiatherogenic HDL to the proatherogenic lipoproteins.[4] Recent studies have also shown that the expression of CETP in transgenic mice significantly increased the formation of atherosclerotic lesions in comparison with control animals, which normally lack plasma cholesteryl ester transfer activity.[5] It is widely accepted that specific inhibitors of the plasma CETP might be good candidates to develop effective therapeutic agents for the treatment of atherosclerotic cardiovascular diseases. In the course of our screening program for CETP inhibitors from microbial sources, we discovered, *via* screening using an Amersham CETP [^{3}H] Scintillation Proximity Assay[6] kit for CETP inhibitors, CETP inhibitors in the cultured broth of a fungal strain. The inhibitors were identified as rosenonolactone analogues. ^{13}C NMR assignments and biosynthetic studies of the rosane diterpenoids were reported by DOCKERILL and HANSON.[7,8] This paper reports the inhibition of CETP by rosenonolactone analogues.

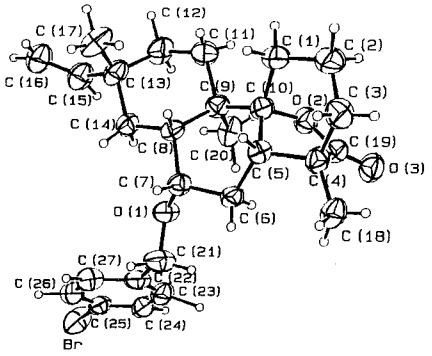
Rosenonolactone analogues (Fig. 1) were isolated from the culture of fungus strain F1064, that was isolated from a soil sample collected at Mt. Jiree, Kyungnam Province, Korea. It was taxonomically identified as *Trichothecium roseum* by the method of PITT and HOCKING.[9] A 2-ml portion of the seed culture was inoculated into a 1-liter baffled flask with four baffles containing 100 ml of production medium (soluble starch 2%, soytone 0.4%, pharmamedia 0.3%, K_2HPO_4 0.1%, $MgSO_4 \cdot 7H_2O$ 0.05%, NaCl 0.2%, $CaCO_3$ 0.3%, $FeSO_4 \cdot 7H_2O$ 0.002%, $MnCl_2 \cdot 4H_2O$ 0.001%, $ZnSO_4 \cdot 7H_2O$ 0.01%, $CoCl_2$ 0.005%). The fermentation was carried out for 5 days at 26°C on a rotary shaker at 150 rpm. After fermentation at 26°C for 5 days, the whole broth (800 ml) was centrifuged to obtain the mycelial part which was extracted with 900 ml of acetone. After filtration and concentration of the acetone extract, the resulting aqueous solution (100 ml) was extracted twice with 500 ml of chloroform. The extracts were concentrated *in vacuo* to dryness to give brown oily materials (1.5 g). The oily material was applied to a silica gel column (30 g). The materials were eluted with a stepwise gradient of *n*-hexane-ethyl acetate. Active fractions were concentrated *in vacuo* to yield pale brown oily material (300 mg). Further purification of the samples was carried out by preparative TLC to yield compound **1** (12.5 mg), compound **2** (5.3 mg) and compound **3** (32.5 mg). The amount of rosenonolactone analogues were determined by silica gel TLC (Merck Art. 5715) with *n*-hexane - ethyl acetate (4 : 6) after extraction of centrifuged mycelium with acetone and silica gel column chromatography. The Rf values of rosenonolactone analogues, **1**, **2** and **3** were 0.37, 0.85, 0.5, respectively. The compounds were identified as the known metabolites rosololactone (**1**), 7-deoxyrosenonolactone (**2**)[10], and rosenonolactone (**3**) according to spectroscopic data (UV, IR, NMR and HR-MS) and X-ray crystallography of synthetic analogue **5**. Compound **4**, which was identified as rosenololactone,[8] was prepared by the reduction of compound **3** with $NaBH_4$ in THF for the study of structure-activity relationship. Benzyl derivative **5** was prepared from **4** for the determination of absolute stereochemistry of rosenonolactone analogues by X-ray crystallograpy. Compound **5** was synthesized by the reaction of 4-bromobenzyl bromide with rosenololactone in the pres-

Fig. 1. Chemical structure of rosenonolactone analogues.



1: R_1=H, R_2=H, R_3=OH **4**: R_1=H, R_2=OH, R_3=H
2: R_1=H, R_2=H, R_3=H **5**: R_1=H, R_2=4-Bromobenzyloxy, R_3=H
3: R_1, R_2=O, R_3=H

Fig. 2. The ORTEP drawing of compound **5**.



ence of K_2CO_3 in CH_3CN at 40°C and purified by silica gel column chromatography. The physico-chemical properties of **5** were as follows: mp 154.3~155.5°C; IR(KBr) cm^{-1} 2934, 1758, 1605, 1458, 1182, 1122, 930. The molecular formula of **5** was established by HR-MS as $C_{27}H_{35}O_3Br$ (*m/z* 487.1832 $[M+H]^+$, calc. 487.1848). Compound **5** was recrystallized from acetone-hexane solution to give colorless crytals. The ORTEP drawing of **5** is shown in Fig. 2.

CETP assays were carried out using an Amersham CETP [^3H] SPA kit and human CETP. Fractions containing CETP activity were isolated from human plasma and dialyzed against 50 mM Tris-HCl buffer (pH 7.4, 150 mM NaCl, 2 mM EDTA). Aliquots were stored at −20°C and used as a source of the CETP. In the CETP-SPA, the transfer of [^3H] cholesteryl esters from HDL to biotinylated LDL is measured following incubation of donor ([^3H] CE-HDL) and acceptor (biotinylated LDL) particles in the presence of human plasma CETP. The assay was carried out in Eppendorf tubes. Reagents were kept on ice prior to setting up the assay. The reaction mixture, containing 10 μl test samples or control vehicle (H_2O or MeOH), 10 μl of 50 mM Hepes buffer (pH 7.4, 0.15 M NaCl, 0.1% (w/v) BSA, 0.05% (w/v) NaN_3), 10 μl of [^3H] cholesteryl ester-HDL (25 μCi) and 10 μl of biotinylated LDL was mixed well. The reaction was initiated by the addition of 10 μl of human CETP. After 4 hours of incubation at 37°C, the reaction was terminated by the addition of 200 μl of streptavidin SPA beads formulated in an assay termination buffer. The terminated Eppendorf tubes were incubated at room temperature for 1 hour to allow the assay to come to equilibrium. Transfer was measured by scintillation counting (Packard Delta-2000) with window settings fully open. Background values were obtained by the addition of H_2O instead of CETP. Percent inhibition of CETP activity is calculated by substracting the background values from both control and test sample values.

$$\% \text{ Inhibition} = 100 \times \left[1 - \frac{\text{Sample (cpm)} - \text{Background (cpm)}}{\text{Control (cpm)} - \text{Background (cpm)}} \right]$$

Rosololactone, 7-deoxyrosenonolactone and rosenololactone mildly inhibited CETP with IC_{50} values 60, 31 and 65 μg/ml, respectively. Rosenonolactone at 300 μg/ml inhibited CETP by 50%. In the structures of rosenonolactone analogues, a ketone residue at C-7 and methylene residue at C-6 (**3**, IC_{50} 300 μg/ml) or methylene residue at C-7 and hydroxyl residue at C-6 (**1**, IC_{50} 60 μg/ml) gave decreased CETP inhibitory activity in comparsion with methylene residues at C-7 and C-6 (**2**, IC_{50} 31 μg/ml) of 7-deoxyrosenonolactone. The CETP inhibitory activity of rosenololactone (**4**, IC_{50} 65 μg/ml), and **5** (IC_{50} 85 μg/ml), which were synthetic compounds, were similar to that of rosololactone (**1**). Rosenonolactone analogues appear to be good models for the design of new CETP inhibitors.

Acknowledgments

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† The sterochemistry of **2**, which was published in this reference, is incorrect. The stereochemistry of rosenonolactone derivatives, which is shown in this paper, is correct.