COMMUNICATIONS TO THE EDITOR

14-(2′,3′,5′-Trihydroxyphenyl)tetradecan-2-ol, a Novel Acetylcholinesterase Inhibitor from *Chrysosporium* sp.

Sir

As a part of the screening program for the isolation of novel microbial metabolites for acetylcholinesterase (AChE) inhibition, we have found that, inhibition shown by 14-(2′,3′,5′-trihydroxyphenyl)tetradecan-2-ol (**1**) from crude extract of *Chrysosporium* sp. is promising. An exhaustive literature survey revealed that this is the first report of this inhibitor from *Chrysosporium* sp. This communication deals with production, isolation, physico-chemical properties and biological activity of the purified inhibitor.

The microorganism, *Chrysosporium* sp., producing the inhibitor collected from imperfect deutromycetes, and is deposited at the Central Food Technological Research Institute Culture Collection Center, Mysore, India, as No. 1106.

The slant culture of the producing organism was inoculated into a 500 ml conical flask containing 100 ml of potato dextrose broth. After incubation at 30°C for 3 days at 250 rpm, the pellets were aseptically transferred to solid state medium consisting of 30 g wheat bran, 30 ml of 0.2 N HCl, 30 ml of distilled water (containing 2.1 mg ferrous sulphate, 2.1 mg copper sulphate and 2.1 mg of zinc sulphate) in 500 ml conical flask. The solid medium was autoclaved for 60 minutes and cooled to room temperature before inoculation. Fermentation was carried for 120 hours in an incubator at 30°C keeping the flasks in a slanting position. Solid state fermentation was carried out in 35 Erlenmeyer flasks.

The fermented wheat bran was extracted exhaustively with 1.8 liter ethyl acetate and distilled to give 8 g of reddish-brown oily material. This was redissolved in ethyl acetate and the solution was washed successively with 5% sodium bicarbonate, 5% sodium carbonate and 5% sodium hydroxide solutions. The above three alkaline extracts were acidified with 2~3 drops of dilute HCl and then extracted with ethyl acetate to give fractions weighing 64 mg, 1.8 g and 90 mg respectively. Evaporation of the remaining ethyl acetate not extracted with the base solutions, gave 2 g residue. All these fractions were tested for AChE inhibition by ELLMAN's method[1]. Only carbonate and bicarbonate washings were found to be active. The combined active fractions were chromatographed on silica-gel column (100~200 mesh) with (8 : 2) chloroform - methanol as mobile phase. The active fraction (200 mg) was washed with 10 ml of benzene. The benzene insoluble was redissolved in 10 ml methanol and boiled for 2 minutes with 50 mg of activated carbon and filtered. The filtrate was concentrated to give 80 mg of a white powder.

The following characteristics were determined for the isolated compound:

The molecular formula of the inhibitor was elucidated as $C_{20}H_{34}O_4$. Molecular weight; 338, MP; 118°C. UV Spectrometry; λ_{max}^{MeoH} nm (ε) 228 (47,300), 257 (27,000), 300 (13,500). Solubility; highly soluble in ethyl acetate and methanol. Nuclear Magnetic Resonance Spectroscopy; ^1H NMR (500 MHz, DMSO-d_6) δ 1.01 (3H, d, 6.2 Hz, CH_3), 1.23 (2H, m, $10CH_2$), 1.34 (2H, CH_2), 2.33 (2H, $-CH_2-Ar$), 3.52 (1H, –CH–O), 5.99 (2H, –Ar–H).

Fig. 1. Structure of 14-(2′,3′,5′-Trihydroxyphenyl)tetradecan-2-ol (**1**).

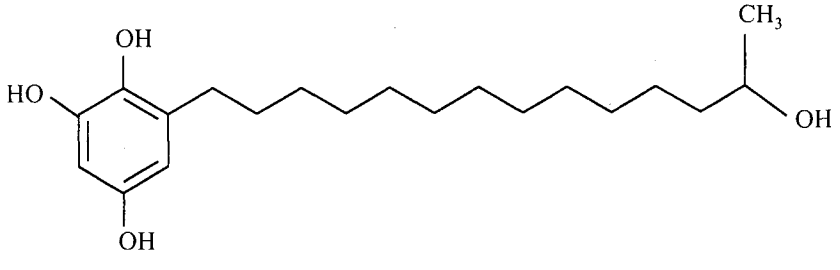

Table 1. Comparative activity shown by the inhibitor isolated from *Chrysosporium* sp. and synthetic neostigmine on AChE from different sources.

Compound	IC_{50} (μM)		
	Human serum AChE	AChE from Rat brain homogenate	Electric eel AChE
14, (2',3',5', Trihydroxyphenyl) tetradecan-2-ol[a)]	197	195	231
Neostigmine	20	1.25	20

[a)] From crude extract of this work.

Mass Spectrometry; EI-MS (*m/z*) 336 (M−2H), 137 (trihydroxyphenyl CH_2–2H), 124 (trihydroxyphenyl), 45 (–CH_3–CHOH). From ^{1}H NMR and GC-MS the structure of the inhibitor was deduced to be 14-(2′,3′,5′-trihydroxyphenyl)tetradecan-2-ol (Fig. 1).

From the literature, *Chrysosporium* sp. was found to produce questin, questinol and asterric acid[2)]. But they were not reported to show inhibition on AChE. Territrem B, territrem C and arisugacin[3)] have been reported as inhibitors of human erythrocyte AChE. Tacrine is a potential inhibitor of AChE in the treatment of Alzheimer's disease[4)]. The inhibitory activity of the purified inhibitor was compared with the inhibition shown by neostigmine against crude AChE from human serum and rat brain homogenate and pure electric eel AChE (Sigma) are shown in Table 1. The inhibitory activities were measured by a modified method of ELLMAN[1)]. Neostigmine has a very high inhibition against AChE from rat brain as compared with AChE from human serum and electric eel. Unlike neostigmine, **1** is equipotent against AChE from various sources. This indicates that, the inhibition shown by the purified inhibitor may possibly have species non-specific mode of action. Even though the purified inhibitor was not found to be potent or selective against AChE it can act as a useful tool for the development of new drugs for Alzheimer's disease.

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