NEW CHLORINE-CONTAINING PRENYLATED C_6-C_3 COMPOUNDS INCREASING CHOLINE ACETYLTRANSFERASE (ChAT) ACTIVITY IN CULTURE OF POSTNATAL RAT SEPTAL NEURONS FROM *ILLICIUM TASHIROI*

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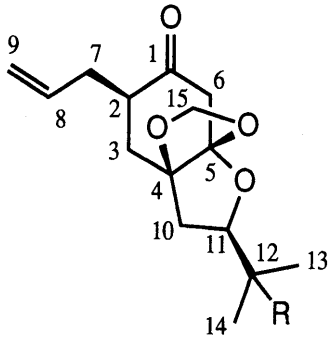
The structures of two new chlorine-containing C_6-C_3 compounds isolated from the woods of *Illicium tashiroi* have been established as 2(*R*)-12-chloro-2,3-dihydroillicinone E (**1**) and 12-chloroillicinone E (**2**) by X-ray crystallographic analysis and spectroscopic data, respectively. Compound **1** has been found to significantly increase ChAT activity in culture of P10 rat septal neurons.

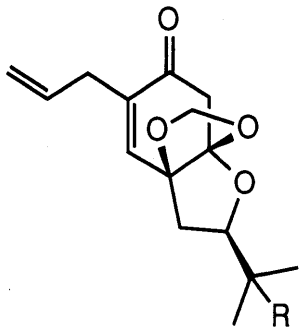
KEYWORDS *Illicium tashiroi*; Magnoliaceae; 2(*R*)-12-chloro-2,3-dihydroillicinone E; 12-chloroillicinone E; prenylated C_6-C_3 compound; choline acetyltransferase activity

Since degeneration of the cholinergic neurons, considered one of the symptoms characteristic of Alzheimer's disease, results in markedly reducing the level of the enzyme choline acetyltransferase (ChAT) activity which catalyzes the synthesis of acetylcholine from its precursor, exogenous neurotrophic substances like nerve growth factor (NGF) which can increase ChAT activity may serve as palliative agents in the treatment of the disease. We have already reported a couple of unique natural products exhibiting such neurotrophic activity in culture of fetal rat cerebral hemispheres.[3] In this communication, we wish to report the structure of a different type of neurotrophic substance, prenylated C_6-C_3 compounds **1** and **2**, containing a chlorine atom isolated from the methanol extract of the wood of *Illicium tashiroi.*

Compound **1**[4] showed a pair of molecular ion peaks at *m/z* 300 and 302 in a 3 : 1 ratio on the electron impact mass spectrum (EI-MS), indicating the presence of a chlorine atom in the molecule. The spectral data of **1** indicated the presence of a ketone (1710 cm^{-1}; δ_C 208.1), an allyl moiety, a methylenedioxy group [δ_H 5.10 (2H, s); δ_C 93.0 (d)], an isolated methylene [δ_H 2.86 (d, 15.1 Hz), 2.90 (d, 15.1 Hz); δ_C 47.4 (d)], and an ABX proton system [δ_H 2.25 (dd, 14.2, 6.5 Hz), 2.45 (dd, 14.2, 10.2 Hz), 3.79 (dd, 10.2, 6.5 Hz)] as well as of a dimethylchloromethyl unit [δ_H 1.53 (s), 1.61 (s); δ_C 27.9 (q), 29.7 (q), 69.9 (s)], which was supported by the observation of a base ion peak at *m/z* 223 derived by a loss of $[Me_2CCl]^+$. The above spectral evidence discloses that **1** is a 12-chlorinated and 2,3-dihydrogenated derivative of illicinone E (**3**)[5] previously isolated from the same source. This was confirmed by a single crystal x-ray analysis.[6] The molecular structure and



1 R = Cl
4 R = OCH_3



2 R = Cl
3 R = OH

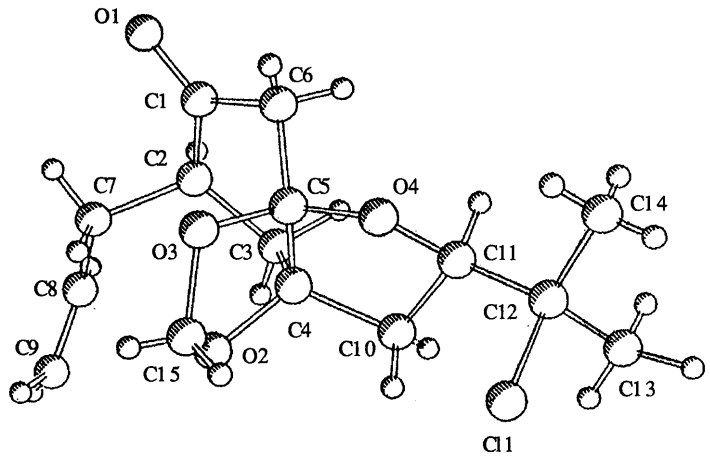


Fig. 1. ORTEP Drawing of **1**

Table I. ^{13}C NMR (100 MHz, $CDCl_3$) Data[a] of **1** and **2**

C	**1**	**2**
1	208.1	194.3
2	44.0	137.6
3	34.0	139.4
4	87.9	85.1
5	111.6	111.0
6	47.4	44.3
7	35.0	33.1
8	135.1	143.2
9	118.0	117.8
10	38.4	38.0
11	83.9	83.6
12	69.9	69.6
13	29.7	29.9
14	27.9	27.2
15	93.0	94.7

a) Assignment was made by HMQC and HMBC.

relative stereochemistry of **1** is shown in the Fig. 1. The absolute configuration for **1** was established to be identical to that of **4**[5] since the CD spectrum of **1** showed the same positive sign at 287 nm as that at 288 nm in **4**. Thus, the structure of **1** was determined as 2(*R*)-12-chloro-2,3-dihydroillicinone E.

Compound **2**[7] had molecular formula $C_{15}H_{19}O_4Cl$ 2 H less than that of **1** and absorption bands attributable to an α,β-conjugated ketone moiety (1680 and 1640 cm^{-1}; 227 nm). This suggests that **2** is a 2,3-dehydrogenated derivative of **1**. In fact, the ^1H- and ^{13}C-NMR[7] (Table I) data of **2** contained the signals assignable to the plane structure **2** which was obtained by 2D NMR analyses (DQFCOSY and HMBC). As concerns the relative stereochemistry of **2**, the methylenedioxy group and the dimethylchloromethyl unit attached at C-11 turn out to take a *syn* relationship to each other from the following NOEs: selective irradiation of the methyl signal at δ_H 1.63 and the H-11

signal (δ_H 3.96) caused distinct NOE enhancements for one (δ_H 5.30) of the methylenedioxy signals and one (δ_H 2.82) of the geminal H-6 protons, respectively. The absolute stereochemistry of **2** was assigned to be identical to that of illicinone E (**3**) on the basis of the same positive Cotton effect at 322 nm for **1** and 320 nm for **3**. Thus, the structure of **2** was assigned as 12-chloroillicinone E.

2(*R*)-12-chloro-2,3-dihydroillicinone E (**1**) was found to increase ChAT to 228 % at 30 μM activity in culture of P10 rat septal neurons.[8] On the other hand, compounds **2**, **3**, and **4** could not increase ChAT activities at the same concentration. Morphological evaluation of the cultured neurons affected by **1** is now under way.

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3) Y. Fukuyama, N. Shida, M. Kodama, *Planta Med.*, **59**, 181 (1993).
4) Colorless needles: mp 75-77°C; $[\alpha]_D^{20}$ -17.4 (c 1.2, EtOH); EIMS *m/z* (rel. int. %): 302 (M^+, 2), 300 (M^+, 6), 272 (16), 270 (39), 223 (100); HRMS *m/z*: 300.1113 (Cacld for $C_{15}H_{21}O_4Cl^{35}$: 300.1128); IR (FT) cm^{-1}: 1710 (C=O), 1640; CD (EtOH) Δε: +8.03 (287 nm); ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 1.53 (s, 3H, 13-CH_3), 1.61 (s, 3H, 14-CH_3), 2.25 (dd, 1H, *J* = 14.2, 6.5, 10-H), 2.45 (dd, *J* = 14.2, 10.2 Hz, 10-H), 2.45 (m, 4H), 2.60 (m, 1H, 7-H), 2.86 (d, 1H, *J* = 15.1 Hz, 6-H), 2.90 (d, 1H, *J* = 15.1 Hz, 6-H), 3.79 (dd, 1H, *J* = 10.2, 6.5 Hz, 11-H), 5.10 (s, 2H, 15-H_2), 5.10 (dd, 1H, *J* = 10.5, 2.1 Hz, 9-H), 5.13 (dd, 1H, *J* = 17.3, 2.1 Hz, 9-H), 5.72 (ddt, 1H, *J* = 17.3, 10.5, 4.5 Hz, 8-H). The previous numbering system for illicinone E and its congeners[5] should be corrected as in this paper.
5) Y. Fukuyama, N. Shida, T. Sakurai, M. Kodama, *Phytochemistry*, **31**, 3975 (1992).
6) Crystal data: monoclinic, a = 8.396 (2), b = 8.846 (4), c = 10.673 (3) Å, β = 102.12 (2)°, space group $P2_1$, Dcalc = 1.289, MoKα radiation, λ = 0.17069, μ = 2.53 cm^{-1}. Diffraction measurement was made on a Rikagku AFC5S. The structure was solved by direct methods and refined by full-matrix least-squares. Final R = 0.047. X-ray crystallographic analysis of **1** was carried out by Dr. M. Kido, Otsuka Pharmaceutical Co., Ltd., to whom we express our application. Detailed data will be published elsewhere.
7) Amorphous: $[\alpha]_D^{20}$ -19.8 (c 2.2, EtOH); EIMS *m/z* (rel. int. %): 300 (M^+, 6), 298 (M^+, 18), 221 (100), 191 (83): HRMS *m/z*: 298.0961 (Cacld for $C_{15}H_{19}O_4Cl^{35}$: 298.0972); IR (FT) cm^{-1}: 1680 (C=O), 1640 (C=C); UV λmax (EtOH) nm (ε): 227 (10500); CD (EtOH) Δε: +7.2 (322 nm);^{1}H-NMR ($CDCl_3$) δ: 1.57 (s, 3H, 13-CH_3), 1.63 (s, 3H, 14-CH_3), 2.29 (dd, 1H, *J* = 14.2, 7.8 Hz, 10-H), 2.63 (dd, 1H, *J* = 14.2, 7.3 Hz, 10-H), 2.82 (d, 1H, *J* = 16.6 Hz, 6-H), 3.02 (dd, 2H, *J* = 6.8, 1.5 Hz, 7-H_2), 3.15 (d, 1H, *J* = 16.6 Hz, 6-H), 3.96 (dd, *J* = 7.8, 7.3 Hz, 11-H), 5.11 (dd, 1H, *J* = 9.8, 1.5 Hz, 9-H), 5.12 (dd, 1H, *J* = 16.6, 1.5 Hz, 9-H), 5.12 (s, 1H, 15-H), 5.30 (s, 1H, 15-H), 5.80 (t, 1H, *J* = 1.5 Hz, 3-H).
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