GARSUBELLIN A, A NOVEL POLYPRENYLATED PHLOROGLUCIN DERIVATIVE, INCREASING CHOLINE ACETYLTRANSFERASE (ChAT) ACTIVITY IN POSTNATAL RAT SEPTAL NEURON CULTURES

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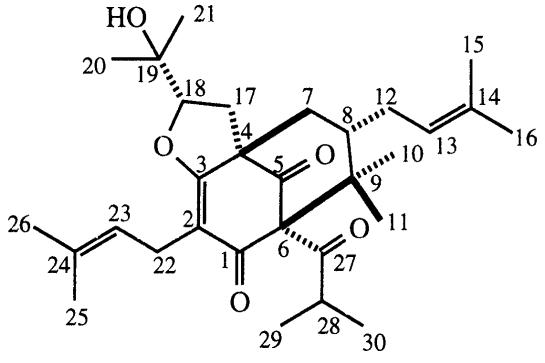
Garsubellin A (**1**), a novel polyprenylated phloroglucin derivative, has been isolated from the wood of *Garcinia subelliptica* and its structure has been elucidated by spectroscopic analyses. Compound **1** could increase the ChAT activity at 10 μM in P10 rat septal neuron cultures.

KEY WORDS *Garcinia subelliptica*; Guttiferae; garsubellin A; polyprenylated phloroglucin; choline acetyltransferase activity

Since a deficiency of choline acetyltransferase (ChAT), which is a key enzyme in the synthesis of the important neurotransmitter acetylcholine, is believed to be implicated in the dementia of Alzheimer disease, a nerve growth factor (NGF)[1] that can not only induce ChAT activity but also promote survival of cholinergic neurons has been anticipated to be a promising agent in the treatment of Alzheimer disease.[2] However, it has the key problems associated with bioavailability and entry into the brain through the blood-brain barrier due to high molecular weight protein. Such drawbacks have compelled us to search for neurotrophic low molecular weight substances[3-5] like NGF in natural products using cultures of fetal rat cerebral hemisphere[6] and/or postnatal rat septal neurons.[7] We wish to report on the structural elucidation of a new polyprenylated phloroglucin derivative **1** named garsubellin A, which was isolated as an inducer of ChAT activity in P10 rat septal neuron cultures from the wood of *Garcinia subelliptica* (Guttiferae).

Garsubellin A (**1**)[8] has a molecular formula $C_{30}H_{44}O_5$ established by high-resolution



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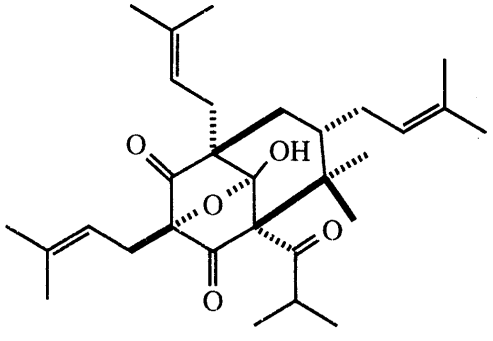

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FABMS indicating nine degrees of unsaturation. The spectral data of **1** revealed the presence of a hydroxyl group (3499 cm^{-1}), two carbonyl groups (1730 cm^{-1}; δ_C 204.7 and 208.5), and a conjugated ketone (268 nm; 1626 cm^{-1}; δ_C 192.9). The ^1H NMR spectrum of **1** has a strong resemblance to that of subellinone (**2**),[2] disclosing that **1** may be a phloroglucin derivative. Extensive analyses of 2D NMR (DQFCOSY and HMQC) data yielded partial structures **A** – **D** in addition to four tertiary methyl groups at δ_H 0.77 (H_3-21), 0.94 (H_3-20), 1.24 (H_3-10), and 1.60 (H_3-11), a tetrasubstituted double bond at δ_C 116.7 (C-2) and 173.2 (C-3), and four quaternary carbons at δ_C 46.6 (C-9), 59.8 (C-4), 70.2 (C-19), and 82.6 (C-6). The HMBC correlations of the H_3-20 and H_3-21 signals with the C-19 and C-18 (δ_C 90.1) signals indicated the presence of a dimethyl carbinol group, which should be linked to the C-18 position of the partial unit **C**. The presence of a cross-peak between the H-18 and C-3 signals which resonated at δ_H 3.92 and δ_C 173.2, respectively, led to a bond formation between C-3 and C-18 via an ether linkage. The remaining tertiary methyl (H_3-10 and H_3-11) signals showed long-range coupling to the C-6 (δ_C 82.6), C-8 (δ_C 43.0), and C-9 (δ_C 46.6) resonances. This means that the C-9 quaternary carbon bearing the H_3-10 and H_3-11 methyl groups must be bonded to C-6, and C-8 in partial unit **A**. Although the C-6 quaternary carbon has no other cross-peak in the HMBC, it should be adjacent to the three carbonyl groups (C-1, C-5, and C-27) to satisfy its low chemical shift value (δ_C 82.6). Following other HMBC correlations between the quaternary carbon and the proton signals of the four partial units, the plane structure shown in Fig. 1 could be devised. The relative configurations at C-4 and C-6 are determined by the bicyclo[3,3,1]nonane framework. Therefore the relative stereochemistry of the chiral carbons at C-8 and C-18 remained to be proved. Judging from the large *J* value (11.3 Hz) for H-8, an isoprepenyl group involved in the unit **A** should take an equatorial orientation at the C-8 position (8*S**).

In a NOESY experiment, H-8 showed a cross-peak to an equatorial proton of H_2-7, which was further correlated to H-18, thereby assigning a C-18 to the *S** configuration. Thus the above spectral data represent the structure of garsubellin A as **1**.

To our knowledge, **1** is the second example of rare polyprenylated phloroglucin derivatives with a tetrahydrofuran ring.[10] Garsubellin A (**1**) could increase ChAT activity by 154% at 10 μM in comparison with a

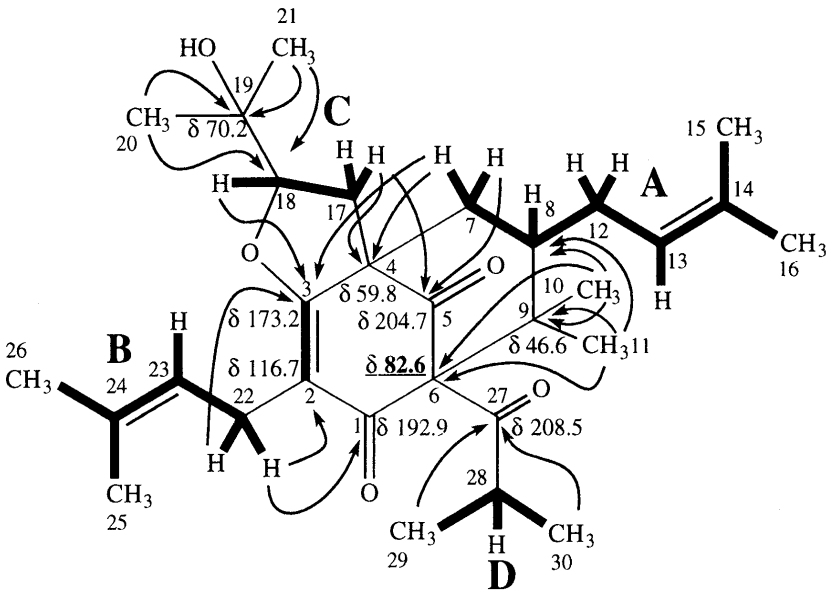

Fig. 1. Bold Lines Indicate Partial Structures (**A** – **D**) Inferred from DQFCOSY and HMQC. Arrows denote the HMBC (J_{C-H} = 8.1 Hz) correlation between the proton (tail) and carbons (head). ^{13}C NMR data (δ_C; 100 MHz in C_6D_6) for the quaternary carbons.

control culture containing 0.5% EtOH in culture of P10 rat septal neurons, as shown in Fig. 2. Its potency is about three times as strong as that of the previously reported tricycloillicinone.[11]

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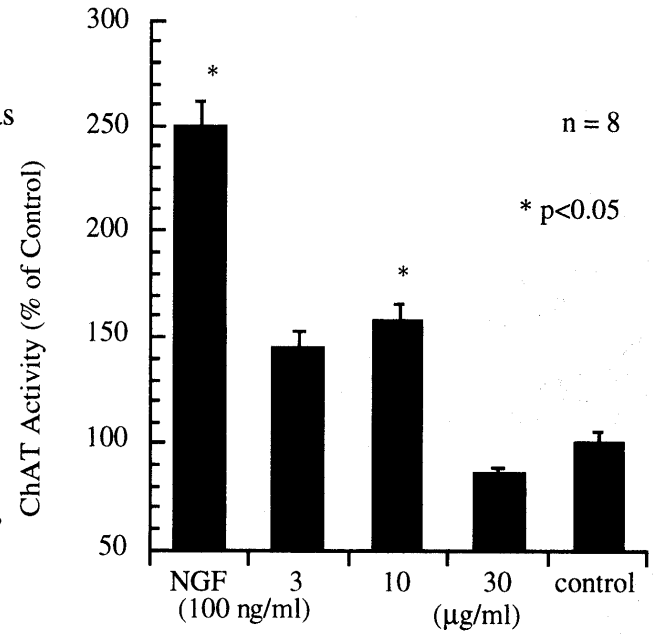

Fig. 2. Effects of Garsubellin A on ChATActivity in Cultures of Postnatal (P-10) Septal Neurons of Rat Basal Forebrain 8 days after Seeding

REFERENCES AND NOTES

1) Thoenen H., Barde Y-A., *Physiol. Rev.*, **60**, 1284-1335 (1980).
2) Hefti F., *J. Neurobiol.*, **25**, 1418-1435 (1994).
3) Fukuyama Y., Kodama M., *Foods & Food Ingredients J. Jpn.*, **169**, 45-56 (1996).
4) Borg J., Toazara J., Hietter H., Henry M., Schmitt G., Luu B., *Febs. Lett.*, **213**, 406-410 (1987).
5) Omura S., Matuzaki K., Fujimoto T., Kosuge S., Fujita S., Nakagawa A., *J. Antibiot.*, **44**, 113-116 (1991).
6) Asou H., Iwasaki N., Hirano S., Dahl D., *Brain Res.*, **332**, 355-357 (1985).
7) Hatanaka H., Tsukui H., Nihonmatsu I., *Dev. Brain Res.*, **39**, 85-95 (1988).
8) **1** (80.5 mg) was isolated from the CH_2Cl_2 -soluble portion (2.6 g). $[\alpha]_D^{20}$ –21.3° (c 1.1, EtOH); HR-FABMS *m/z*: 485.3269 $[M+H]^+$ (Cacld for $C_{30}H_{45}O_5$: 485.3267); IR cm^{-1}: 3499 (OH), 1730 (C=O), 1626 (conj. C=O); UV λmax (EtOH) nm: 268 (ε 16300); ^{1}H-NMR (400 MHz, C_6D_6) δ: 0.77 (s, 3H, 21-CH_3), 0.94 (s, 3H, 20-CH_3), 1.24 (s, 3H, 10-CH_3), 1.30 (d, 3H, *J* = 6.6 Hz, 29-CH_3), 1.30 (dd, 1H, *J* = 13.6, 11.3 Hz, 7-H), 1.32 (dd, 1H, *J* = 12.9, 5.9 Hz, 17-H), 1.37 (d, 3H, *J* = 6.6 Hz, 30-CH_3), 1.45 (s, 3H, 15-CH_3), 1.58 (s, 3H, 16-CH_3), 1.58 (m, 1H, 12-H), 1.60 (s, 3H, 11-CH_3), 1.61 (s, 3H, 26-CH_3), 1.70 (s, 3H, 25-CH_3), 1.74 (dddd, 1H, *J* = 11.3, 7.1, 4.5, 3.6 Hz, 8-H), 1.93 (dd, 1H, *J* = 13.6, 4.5 Hz, 7-H), 2.09 (ddd, 1H, *J* = 13.4, 7.1, 3.6 Hz, 12-H), 2.26 (qq, 1H, *J* = 6.6, 6.6 Hz, 28-H), 2.73 (dd, 1H, *J* = 12.9, 10.7 Hz, 17-H), 3.21 (dd, 1H, *J* = 14.2, 7.3 Hz, 22-H), 3.39 (dd, 1H, *J* = 14.2, 7.1 Hz, 22-H), 3.92 (dd, 1H, *J* = 10.7, 5.9 Hz, 18-H), 4.96 (dd, 1H, *J* = 7.1, 7.1 Hz, 13-H), 5.40 (dd, 1H, *J* = 7.3, 7.1 Hz, 23-H). ^{13}C-NMR (100 MHz, C_6D_6) δ: 16.5 (C-10), 17.8 (C-15), 17.9 (C-25), 20.9 (C-30), 21.9 (C-29), 22.6 (C-22), 23.1 (C-11), 24.4 (C-21), 25.7 (C-26), 25.9 (C-16), 26.3 (C-20), 27.0 (C-12), 30.3 (C-17), 39.0 (C-7), 42.7 (C-28), 43.0 (C-8), 46.6 (C-9), 59.8 (C-4) 70.2 (C-19), 82.6 (C-6), 90.1 (C-18), 116.7 (C-2), 122.0 (C-23), 123.2 (C-13), 132.4 (C-24), 133.2 (C-14), 173.2 (C-3), 192.9 (C-1), 204.7 (C-5), 208.5 (C-27).
9) Fukuyama Y., Kaneshi A., Tani N., Kodama M., *Phytochemistry*, **33**, 483-486 (1993).
10) Wärtgen K., Witchel M., *Planta Med.*, **55**, 628-629 (1989).
11) Fukuyama Y., Shida N., Kodama M., Chaki H., Yugami T., *Chem. Pharm. Bull.*, **43**, 2270-2272 (1995).

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