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Article

5-(Hydroxymethyl)-2-furfuraldehyde, Anticonvulsant Furan from the Arils of *Euphoria longana* L.

Dong-Hyun Kim, Dae-Won Kim[1], Soo-Young Choi[1], Chang-Ho Park[2] and Nam-In Baek*

Graduate School of Biotechnology & Plant Metabolism Research Center, KyungHee University, Suwon 449-701
[1]Department of Genetic Engineering, Division of Life Science, Hallym University, Chunchon 200-702
[2]College of Environment & Applied Chemistry, Kyung Hee University, Suwon 449-701, Korea

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Arils of *Euporia longana* L. was extracted with 80% aqueous MeOH and partitioned successively with EtOAc, *n*-BuOH and H_2O. From the *n*-BuOH fraction, furan compound was isolated through silica gel column chromatography. The results of physico-chemical data including NMR, MS and IR revealed the compound to be 5-(hydroxymethyl)-2-furfuraldehyde. This compound stimulated GDH I activity by 19.2±0.6, 41.2±0.9, 68.4±1.1, 80.3±0.9 and 85.9±1.6% at *in vitro* concentrations of 0.005, 0.008, 0.01, 0.02 and 0.03 %, respectively.

Key words: Anticonvulsant furan, *glutamate dehydrogenase*, *5-(hydroxymethyl)-2-furfuraldehyde*, Euporia longana

γ-Aminobutyric acid (GABA) is present in tissues of mammalians, as well as at competitive amounts in the central nervous system (CNS), where it has been known to be a major inhibitory chemical neurotransmitter.[1)] When the concentration of GABA in brain diminishes to below a threshold level, various neurological disorders including epilepsy, seizures, convulsions, Huntington's disease, and Parkinsonism occur.[2-4)] The concentration of GABA in brain is controlled by an oxidation enzyme, glutamate dehydrogenase (GDH, EC 1.4.1.3), which catalyzes the reversible amination of 2-oxoglutarate to glutamate and the glutamate is converted into GABA through decarboxylation. We have investigated natural sources increasing the activity of GDH. However, natural product showing stimulatory effect of GDH I is very rare although a large number of compounds which inhibit SSAR (succinic semialdehyde reductase) and SSADH (succinic semialdehyde dehydrogenase) concerned with an anticonvulsant effect exist in natural souces.

The fruit of *Euphoria longana* L. (Sapindaceae) with a sweet taste is found in China and South Asia. The arils of this plant (Longan Arillus) have been used as tonic, and for the treatment of amnesia, insomnia and various palpitations due to fright, among others.[5,6)] Several compounds including nucleosids such as adenosine, adenine, uridine, 5-methyluridine[7)] and uracil[6)] and tannin such as corilagin[8)] and acetonylgerain[9)] have been isolated from the arils of *E. longana* L. This deals with the isolation of a furan compound from this plant, which was first isolated from natural source, and the evaluation of the compound for the stimulatory effect on GDH I.

*Corresponding author
Phone: +82-31-201-2661 Fax: +82-31-201-2157
E-mail: nibaek@khu.ac.kr

Materials and Methods

Instruments. EI mass spectrum was taken on a JEOL JMSAX 505-WA spectrometer and IR spectrum on a Perkin Elmer Spectrum One FT-IR spectrometer. The ^{1}H- and ^{13}C-NMR spectra were taken on a Varian Unity Inova AS 400 FT-NMR spectrometer.

Materials. The arils of *E. longana* L. were purchased at a market in Seoul, Korea. A voucher specimen (KHU02174) was reserved at the laboratory of Natural Products Chemistry, KyungHee University, Suwon, Korea.

NADH and 2-oxoglutarate were purchased from Sigma Chemical Co. The GDH isoproteins were purified from bovine brain through the method developed in author's laboratory[10)] and were homogeneous as judged by Coomassie-stained gradient SDS-polyacrylamide gel electrophoresis. All other chemicals and solvents were reagent grade or better.

Isolation of 5-(hydroxymethyl)-2-furfuraldehyde. The dried sample (5 kg) was extracted at r.t. with 80% aqueous MeOH (15 *l*×2) and partitioned successively with water (1 *l*), EtOAc (1 *l*×2) and *n*-BuOH (1 *l*×2). The *n*-BuOH extract (LAB) was subjected to silica gel column chromatograph (c.c.) and eluted with $CHCl_3$-MeOH-H_2O (10 : 3 : 1, lower layer) monitoring by thin layer chromatography (TLC) to produce 13 fractions (LAB1~LAB13).

The first fraction (LAB1, 660 mg) was subjected to silica gel c.c. (ϕ4.5×15 cm) eluted with $CHCl_3$-EtOH (30 : 1) to afford six fractions (LAB1-1~LAB1-6). The obtained third fraction (LAB1-3, 177 mg) was applied to the silica gel c.c. (ϕ4×13 cm) eluted with $CHCl_3$-EtOH (20 : 1) to give sub-fractions (LAB1-3-1~LAB1-3-3) and the third sub-fraction (LAB1-3-3) was subjected to the silica gel c.c. (ϕ3×15 cm) eluted with $CHCl_3$-MeOH (20 : 1) to afford four fractions

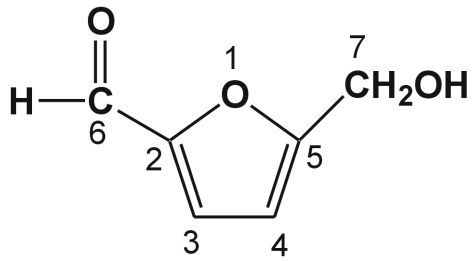

Fig. 1. Chemical structure of compound 1 from the arils of *Euphoria longana* L.

(LAB1-3-3-1~LAB1-3-3-4) the third of which (LAB1-3-3-3, 42) was finally applied to the silica gel c.c. (ϕ3 × 18 cm) eluted with *n*-hexane-EtOAc-BuOH (40 : 20 : 1) to ultimately produce the purified compound **1** (20 mg).

Compound **1** {5-(hydroxymethyl)-2-furfuraldehyde}: Yellow oil, EI/MS *m/z*: 126 (M^+), 109, 97; IR_v ($CHCl_3$, cm^{-1}) 3400, 2850, 1670; ^{1}H-NMR (400 MHz, CD_3OD) 9.53 (1H, s, H-6), 7.38 (1H, d, *J* = 3.4 Hz, H-3), 6.58(1H, d, *J* = 3.4 Hz, H-4), 4.60 (2H, s, H-7); ^{13}C-NMR (100 MHz, CD_3OD) 179.45 (C-6), 163.21 (C-5), 153.90 (C-2), 124.87 (C-3), 110.90 (C-4), 57.61 (C-7).

Stimulatory activity assay on GDH I. GDH I activity was measured spectrophotometrically in the direction of reductive amination of 2-oxoglutarate by following the decrease in absorbance at 340 nm.[10] All assays were performed in triplicate, and initial velocity data were correlated with a standard assay mixture containing 50 mM triethanolamine, pH 8.0, 100 mM ammonium acetate, 0.1 mM NADH, and 2.6 mM EDTA at 25°C. GDH concentrations were adjusted to give a measured rate of less than 0.04 absorbance units per min. The reaction was started with the addition of 2-oxoglutarate to a 10 mM final concentration. One unit of enzyme was defined as the amount of enzyme required to oxidize 1 μmol of NADH per min at 25°C. Stimulation studies were performed at various concentrations (0.005%, 0.008%, 0.01%, 0.02% and 0.03%) in assay buffer at 25°C as described in the reference.[11]

Results and Discussion

The arils of *E. longana* L. were exracted with MeOH and sequentially partitioned with EtOAc, *n*-BuOH and water. Activity-guided fractionation using repeated silica gel c.c. of the *n*-BuOH fraction led to the isolation of an active furan compound (**1**).

Compound **1** was obtained as yellow oil in $CHCl_3$-MeOH, and showed absorbance bands at 3400 and 1670^{-1} in the IR spectrum ($CHCl_3$) due to hydroxyl and double bond, respectively. The ^{13}C-NMR spectrum of **1** exhibited one aldehyde (δ179.45), two oxygenated olefinic quaternary (δ163.21, δ153.90), two olefinic methine (δ124.87, δ110.90) and one oxygenated methylene (δ57.61) carbon signals. In addition, in the ^{1}H-NMR spectrum of **1**, one aldehyde at

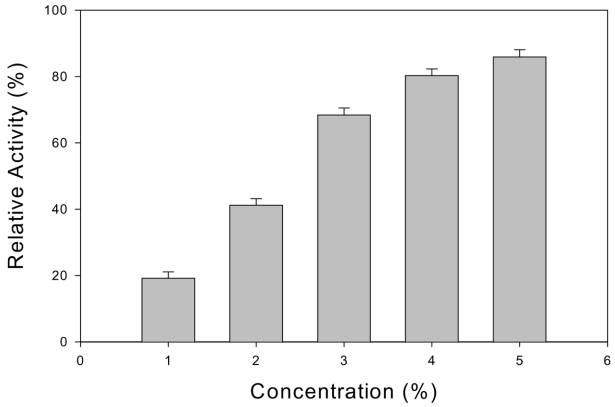

Fig. 2. Stimulatory effect of 5-(hydroxymethyl)-2-furfuraldehyde on GDH I. Relative activities are expressed as percentage of the control. Sample concentrations were 0.005 (1), 0.008 (2), 0.01 (3), 0.02 (4) and 0.03% (5). Experiments were carried out in triplicates and vertical bars indicate means ± S.D.

δ9.53(1H, s), two olefinic methine at δ7.38(1H, d, *J* = 3.4 Hz) and δ6.58(1H, d, *J* = 3.4 Hz), which indicated the presence of a furan ring, and one oxygenated methylene at δ4.60(2H, s) were observed. Through comparison of NMR data with reference,[12] compound **1** was identified as 5-(hydroxymethyl)-2-furfuraldehyde, an active constituent of Hachimi-jio-gan, showing a potent inhibitory effect on aldose reductase.[12] This compound was isolated for the first time from *E. longana* L.

5-(hydroxymethyl)-2-furfuraldehyde was examined for the stimulatory effects on GDH I at various concentrations. The compound concentration-dependently increased the activities of GDH I (Fig. 2). Although the stimulatory effect of 5-(hydroxymethyl)-2-furfuraldehyde on GDH I was lower than that of gabapentin[11], which was reported as the synthetic positive control for this activity, it is still of value that this GDH I active compound was isolated from a natural source.

The stimulatory effect of 5-(hydroxymethyl)-2-furfuraldehyde on GDH I may result in the elevation of neurotransmitter GABA levels in CNS. Therefore, *E. longana* L. may be clinically useful for the treatment of various neurological disorders including epilepsy, seizures, convulsions, Huntington's disease, and Parkinsonism. The successive studies including *in vivo* test are now under way.

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