AN ANTIVITAMIN B$_6$, 4'-METHOXYPYRIDOXINE,
FROM THE SEED OF GINKGO BILOBA L.

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An antivitamin B$_6$, 4'-methoxypyridoxine (1) was isolated from the
seed of Ginkgo biloba L. (Ginkgoaceae). The albumen of the seed of
G. biloba L., which is called "Gin-nan or Ginkyo", is used as a crude
drug in China and as food in Japan. However, in Japan, there have been
about 70 reports of "Gin-nan sitotoxism."

In this paper, we report that the substance responsible for this
sitotoxism is 4'-methoxypyridoxine (1), which is known to have anti-
vitamin B$_6$ activities. This compound (1) is reported for the first
time from natural products.

Keywords — Ginkgo biloba L.; Ginkgoaceae; antivitamin B$_6$;
4'-methoxypyridoxine; Gin-nan sitotoxism; convulsive agent;
acute toxicity; guinea pig

"Gin-nan" is the seed of Ginkgo biloba L. (maidenhair tree, Ginkgoaceae), and
its albumen is used as a crude drug in China and as food in Japan. "Gin-nan sitotoxism"
(cardinal symptom: convulsions) has sometimes occurred during food shortages
in Japan (1930-1960). Mainly infants contracted this sitotoxism (lethality: about
27%). Several investigations have been undertaken to elucidate the cause of the
sitotoxism, but the cause of it remained unknown. This paper describes our
finding that 4'-methoxypyridoxine (1), antivitamin B$_6$, isolated from G. biloba is
the substance that causes "Gin-nan sitotoxism."

The albumen of Ginkgo seeds was dried at 40°C for 7 days and then ground. The
powder was treated as reported before. The outline of the procedure is shown in
Chart 1. The toxicity was evaluated by orally administered acute toxicity tests
using guinea pigs (250-400 g) of either sex. Test samples were dissolved or sus-
pended in water in appropriate concentrations. Signs of toxicity were paralysis of
legs, opisthotonus, tonic convulsions, and auditory hyperalgesia. These symptoms
occurred within 30-40 minutes after administration of the toxic fractions.

The isolation procedure afforded compound (1) [ca. 0.01% yield, dry weight],
which induced the characteristic convulsions in guinea pigs at an oral dose of 11
mg/kg.
dried albumen (Ginkgo biloba L.)
H₂O
filtrate
centrifuged, 2000 rpm, 10 min

precipitate [-] supernatant [+ : 38 ml/kg]
1) 80°C, 1 h
2) precipitated with EtOH

residue [-] soluble portion [+ : 4.3 g/kg]
1) concentrated
2) petroleum ether

p. ether layer [-] aqueous layer [+ : 2.9 g/kg]
AcOEt

AcOEt layer [-] aqueous layer [+ : 1.1 g/kg]
BuOH

aqueous layer [-]
BuOH layer [+ : 190 mg/kg]
Al₂O₃ column chromatog.
CHCl₃-MeOH (19:1, v/v)
compound (1) [+ : 11 mg/kg]

Chart 1

[+ : ml, g, or mg/kg] indicates toxic fraction and dose.

(Typical symptoms for this sitotoxism occurred with this dose.)

[- ] indicates nontoxic fraction.

(In this fraction, no typical symptoms occurred when administration dose was more than that of corresponding toxic fraction.)

Compound (1) was found by high resolution mass spectrum to have the molecular formula C₉H₁₃NO₃ [183 (M⁺, C₉H₁₃NO₃, Calcd: 183.0891. Found: 183.0890)]. Compound (1) showed positive reactions with ferric chloride solution, and with Fast Blue B Salt (diazonium reagent) indicating that it has a hydroxypridine without substitution groups at the para position. ¹H- and ¹³C-NMR spectra ⁴ showed the presence of an aromatic methyl group [2.42(3H, s, C₂-CH₃) and 18.4(q, C₂-CH₂)], a methoxy group [3.44(3H, s, C₄-OCH₃) and 58.8(q, C₄-OCH₃)], two methylene groups (-CH₂O-) [4.63 and 4.76(each 2H, each s, C₄- and C₅-CH₂O-) and 60.4, 67.9(each t, C₅- and C₄-CH₂O-)], an aromatic proton [7.90(1H, s, C₆-H)], and a tetrasubstituted pyridine ring [130.8(d, C₆), 131.3(s, C₂), 135.2(s, C₄), 147.6(s, C₂), and 152.4(s, C₃)].

As compound (1) coupled with 2,6-dichloroquinochloroimide (Gibbs reagent) even in the presence of borate,⁵ it was a 4'-substituted (i.e. 4'-methoxy) pyridoxygen. The mixed melting point with authentic 4'-methoxypryidoxine hydrochloride (mp 181°C)⁶ was 179-180°C. Compound (1) was identified as 4'-methoxypryidoxine (2-methyl-3-hydroxy-4-methoxymethyl-5-hydroxymethyl pyridine) by these analyses.
4'-Methoxypyridoxine is known to have antivitamin B₆ activities and to be a synthetic potent convulsive agent in a variety of animals and in man. However, pyridoxine (vitamin B₆) prevents the convulsions, or promptly stops them. Therefore, pyridoxine may prevent the symptoms of "Gin-nan sitotoxism," because these symptoms are derived from the effect of vitamin B₆ deficiency induced with compound (1). The lack of vitamin B₆ during food shortage times also may be one of the causes of "Gin-nan sitotoxism." Compound (1) (i.e. 4'-methoxypyridoxine) was found for the first time from natural products.

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