Short Communication

Isolation of Zeanoside C, a New Metabolite of IAA from Corn (*Zea mays* L.) Kernels[†]Koichi TATEISHI, Hisao SHIBATA
and Yutaka MATSUSHIMADepartment of Agricultural Chemistry,
Faculty of Agriculture, Shinshu University,
8304 Minamiminowa, Kamiina,
Nagano 399-45, Japan

Received August 26, 1988

In the previous paper,¹⁾ we reported two new metabolites of IAA named zeanoside A and B from immature sweet corn (*Zea mays* L.) kernels, their structures having been elucidated as the 7-*O*- β -D-glucopyranoside of 3,7-dihydroxy-2-indolinone-3-acetic acid and the 8-*O*- β -D-glucopyranoside of 2-quinolone-4-carboxylic acid by chemical studies and spectral data. Further investigation of this kernel resulted in the isolation of a new stereoisomer of zeanoside A.

In this paper, we describe the isolation and structural elucidation of this new compound named zeanoside C.

Dent corn (cv. Pioneer and White) and sweet corn (cv. Golden Cross Bantam and Honey Bantam), which were grown on an experimental farm at Shinshu University, were harvested 10 to 50 days after silking, and immediately stored at -20°C . Kernels (3 kg) of the Pioneer dent corn (yellow) were ground with a solution of conc. HCl (45 ml) in *n*-butanol (9 l) using an ordinary grinder, and then filtered. The acidic filtrate was centrifuged, and the supernatant (*n*-butanol layer) was extracted with saturated aqueous sodium bicarbonate, which was then washed with *n*-butanol three times. The aqueous solution was adjusted to pH 6 with 1 *N* HCl, and then concentrated *in vacuo*. The concentrate was

further adjusted to pH 2 with 1 *N* HCl. The solution was adsorbed on a charcoal column, which was washed with water (20 l) and then eluted with a mixture of ethanol, water and NH_4OH (50:45:5, v/v). The eluate (3 l) was concentrated *in vacuo* to a small volume. The concentrate (20 ml) was charged to an Amberlite IRA-400 (Cl^-) column, which was washed with water (20 l) and successively eluted with 0.03 *N* HCl (3 l). The eluate was treated with charcoal by the same method as that already mentioned. The fraction eluted with a mixture of ethanol, water and NH_4OH (50:45:5, v/v) was further purified by repeated paper chromatography (*n*-butanol-acetic acid-water=4:1:5, v/v, developed 3 times), using Toyo Roshi No. 50. The dried paper showed seven main bands detected under a UV lamp (λ_{254} and 366 nm).

The fourth fraction of the paper showed a pair of peaks at 248 and 295 nm of its UV spectrum, and its hydrolysate (1 *N* HCl, 100°C , 2 hr) was identical with zeanic acid.²⁾

This band was collected and extracted with 50% aqueous ethanol, before the extract was evaporated to afford a syrup (30 mg). TLC analysis of this syrup showed a single spot, but the chromatogram from HPLC gave two major peaks (t_R 16.1 min, which corresponds to zeanoside A, and 17.7 min).

This material (30 mg) was further separated into two fractions by HPLC on an Inertsil ODS 5 column (10.7×250 mm), using 0.05 *M* ammonium acetate, under the following conditions: detection, absorbance at 257 nm or 295 nm; flow rate, 3 ml/min; amount of injection, 1 mg/50 μl H_2O . The compound corresponding to the elution time of 17.7 min was frozen at -20°C , and then lyophilized to yield an amorphous solid (5.0 mg). This material showed only a single peak with a retention time of 17.7 min under the same HPLC conditions.

Recrystallization of this compound from ethanol afforded fine crystals of zeanoside C (3.7 mg).

[†] Studies on IAA Related Compounds. Part II. For Part I, see ref. 1.

FIG. 1. Structures of Zeanoside A and C.

The result from HPLC analysis showed that the kernels of all cultivars contained zeanoside A, B and C, the concentration of which was different among the four cultivars.

Properties of zeanoside C. mp 162~163°C. $[\alpha]_D^{19} -162.1^\circ$ ($c=0.1$, H_2O). UV λ_{max} (H_2O) nm (ϵ): 248 (4700), 295 (2700). UV λ_{max} (1N HCl) nm (ϵ): 248 (4700), 295 (2700). UV λ_{max} (1N NaOH) nm (δ): 245 (6000), 265 (sh, 5800), 300 (2400). IR λ_{max} (KBr) cm^{-1} : 3390 (OH), 3200~2500 (OH, COOH), 1705 (C=O), 1639 (C=N), 1580, 1287, 1223, 1199, 1074~1022 (pyranose ring), 868. MS (SIMS, M+H) m/z : 386 ($C_{16}H_{19}O_{10}N+H$), 368, 324, 224, 162.

An acid treatment (2N HCl, 100°C, 2 hr) of zeanoside C and zeanoside B¹⁾ gave zeanic acid and glucose. The IR spectrum of zeanic acid obtained agreed with that of the authentic material described by Matsushima *et al.*²⁾

The formation of glucose was recognized by PPC analysis (*n*-butanol-acetic acid-water = 4:1:5, v/v, developed 3 times) and by GC analysis³⁾ (3% ECNSS-M, column, 2 mm $\times$ 2 m, 190°C) after $NaBH_4$ reduction of the glucose and successive acetylation. Its t_R (57.3 min) coincided with that of authentic sorbitol hexaacetate.

From these facts, zeanoside C was found to be a glucoside of zeanic acid or one of its related compound. The aglycon, however, was not zeanic acid, since the MS (SIMS) spectrum of zeanoside C showed the pseudo-molecular ion peak at m/z 386 ($C_{16}H_{17}O_9N+H$) instead of at m/z 368 [$C_{16}H_{17}O_{10}N+H$] (CI-MS) for zeanoside B.⁴⁾ It is well known that 3-hydroxy-2-indolinone-3-acetic acid can be easily converted into a 4-carboxy-2-quinolone⁵⁾ by acid treatment. From these facts, the structure of

the aglycon of zeanoside C was presumed to be 3,7-dihydroxy-2-indolinone-3-acetic acid.

The structure of zeanoside C was elucidated from its NMR spectra in $DMSO-d_6$, using tetramethylsilane as an internal standard.

The 1H NMR spectrum of zeanoside C showed signals due to a methylene group (δ 2.89, 2H, s) adjacent to a carboxyl group, a methine group on the aromatic ring (δ 6.84, 1H, d, $J=10$ Hz, C_5-H ; δ 7.02, 1H, d, $J=10$ Hz, C_4-H ; δ 7.10, 1H, d, $J=10$ Hz, C_6-H), and a carboxyl group (δ 10.01, br.). In addition to these signals, signals due to a glucose moiety were observed (δ 3.15, C_5-H ; δ 3.26, C_6-H ; δ 3.32, C_4-H ; δ 3.44, C_3-H ; δ 3.71, bd, $J=7$ Hz, C_2-H ; δ 4.65, C_1-H). The ^{13}C NMR spectrum showed signals due to the aglycon at δ 40.3 ($C\alpha$, $-CH_2-$), δ 73.14 (C_3 , $-CH-O-$), δ 116.89 (C_4 , C_5 or C_6 , $CH=$), δ 117.85 (C_4 , C_5 or C_6 , $CH=$), δ 121.91 (C_4 , C_5 or C_6 , $CH=$), δ 131.96 (C_{4a} , C_7 or C_{7a} , $C=$), δ 141.43 (C_{4a} , C_7 or C_{7a} , $C=$), δ 170.07 (COOH) and δ 177.81 (C_2 , $C=O$), and due to a sugar moiety at δ 60.71 (C_6 , $-CH_2-$), δ 69.77 (C_5 , $-CH-$), δ 73.36 (C_4 , $-CH-$), δ 75.54 (C_2 , $-CH-$), δ 77.14 (C_3 , $-CH-$) and δ 102.54 (C_1 , $-CH-$).

As zeanoside C has a quaternary carbon atom bearing an oxygen atom and an acetic acid moiety, a reasonable structure for the aglycon was concluded to be 3,7-dihydroxy-2-indolinone-3-acetic acid.

On the 1H NMR spectrum, the coupling constant of the anomeric proton (δ 4.56 ppm) of the glucosyl moiety was 6.9 Hz, and on the ^{13}C NMR spectrum, the anomeric carbon signal appeared at 102.54 ppm. These data show that the sugar moiety in zeanoside C was β -glucosyl.^{6,7)}

From these spectroscopic analyses, zeano-

side C was elucidated to 7-*O*- β -D-glucopyranosyl 3,7-dihydroxy-2-indolinone-3-acetic acid (**I** or **II** in Fig. 1.).

The ^1H and ^{13}C NMR spectra of zeanoside C were similar to those of zeanoside A, in which the values of most peaks were observed at a 0~0.11 ppm (^1H) and 0~0.25 ppm (^{13}C) lower field than those of zeanoside A.

The fragmentation patterns in the mass spectrum of zeanoside C agreed with that of zeanoside A, but clear differences between them were found in the melting point, t_R on HPLC, IR spectrum and $[\alpha]_D$ value (zeanoside A, -36.8° ; zeanoside C, -162.1°).

Consequently, zeanoside C was concluded to be a stereoisomer of zeanoside A at the C_3 position (**I** or **II** in Fig. 1).

Acknowledgments. We express our thanks to Professor M. Nakayama of the Department of Agricultural Chemistry of University of Osaka Prefecture for his invaluable advice and suggestions. We also thank

Professor S. Kurosawa, Dr. K. Sen, Professor R. Iriye and Dr. M. Hirota, of Shinshu University for their valuable suggestions and helpfulness. We also thank Hitachi Seisakusho for MS measurements.

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