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## Note

# Structure of aconitan A, a hypoglycemic glycan of *Aconitum carmichaeli* roots*

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A hypoglycemic principle of *Aconitum carmichaeli* roots, aconitan A, a glycan having a molecular weight of ~8700, is shown by physico-chemical and chemical examinations to be composed of α-(1→6)-linked D-glucopyranose residues with three branching points at O-3.

We have recently isolated four glycans possessing hypoglycemic activity, aconitans A, B, C, and D, from the crude drug "bushi" (aconite), the roots of *Aconitum carmichaeli* Debeaux (Ranunculaceae)[1]. Structural examination of the main polysaccharide, aconitan A, is described in this paper.

## RESULTS AND DISCUSSION

Gel chromatography of aconitan A gave a value of 8700 for the molecular weight. Glucose was identified as the only component and nitrogen was absent. This polysaccharide was strongly dextrorotatory ($[\alpha]_D$ +190°) and its $^1$H-n.m.r. spectrum exhibited an anomeric doublet ($J$ = 3 Hz) at $\delta$ 4.98, demonstrating the D-glucose residues to be α-linked.

Seven major signals visible in the $^{13}$C-n.m.r. spectrum, at $\delta$ 63.2, 68.3, 72.2, 72.9, 74.1, 76.1, and 100.4, were assignable to C-6 (free), C-6 (linked), C-4, C-5, C-2, C-3, and C-1, respectively, indicating that α-D-glucose residues are linked at the 1 and 6 positions. The appreciable intensity of the signal for C-6 carbon having a free hydroxyl group was indicative of branching in aconitan A. However, the branching position could not be deduced from the $^{13}$C-n.m.r. spectrum at this stage, because branching was infrequent.

Aconitan A was methylated with methylsulfinyl carbanion and methyl iodide in dimethyl sulfoxide[2]. The fully methylated product was successively hydrolyzed,

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Chart I. Structural feature of aconitan A. Glcp = glucopyranose. $a + b + c + d + e + f + g = \sim 47$.

reduced, and acetylated. The partially methylated glucitol acetates thus obtained were analyzed by gas–liquid chromatography–mass spectrometry (g.l.c.–m.s.)[3]. Three products were identified: 2,3,4,6-tetra-*O*-methyl-, 2,3,4-tri-*O*-methyl-, and 2,4-di-*O*-methyl-D-glucitol acetates, in the molar ratio of 1.2:9.0:1.0, showing that aconitan A is branched through O-3.

When aconitan A was oxidized with periodate, 1.6 mol of periodate per mol of sugar residues was consumed, with liberation of 0.8 mol of formic acid. The periodate-oxidized product was successively reduced[4], hydrolyzed, and analyzed, the yield of residual glucose being 5.8%.

As it is known that the (1→6)-glucosidic linkage is less stable to acetolysis than the (1→3)-linkage[5], aconitan A was acetolyzed with acetic anhydride–acetic acid–sulfuric acid. The acetolysis product was deacetylated and analyzed by thin-layer chromatography (t.l.c.) and the trimethylsilylated ($Me_3Si$) products were examined by g.l.c. Both glucose and nigerose were detected, and determined to be in the molar ratio of 19:1. No component other than glucose and nigerose was found in the acetolysis product.

From these results, it may be concluded that aconitan A is mainly composed of α-(1→6)-linked D-glucopyranose residues and has branches linked, in part, through O-3. In respect to the degree of branching, the methylation analysis and the Smith degradation show some apparent differences. The acetolysis results better support the value obtained by Smith degradation than does the methylation analysis. Aconitan A is concluded to be composed of ~54 glucose residues having three branching points, as shown in Chart I.

## EXPERIMENTAL

*General methods.* — N.m.r. spectra were measured in $D_2O$. Chemical shifts (δ) are expressed in p.p.m. downfield from sodium 4,4-dimethyl-4-silapentanoate as the internal standard.

*Aconitan A.* — The compound had $[\alpha]_D$ +190° (*c* 0.60, water); $^1$H-n.m.r. δ 4.98 (d, *J* 3 Hz); $^{13}$C-n.m.r.: δ 63.2, 68.3 (C-6), 72.2 (C-4), 72.9 (C-5), 74.1 (C-2), 76.1 (C-3), and 100.4 (C-1).

*Gel chromatography.* — Aconitan A (2 mg) in water was applied to a column (2.6 i.d. × 95 cm) of Sephadex G-75 that was eluted with 0.1M Tris · HCl buffer (pH 7.0); 5-mL fractions were collected and analyzed by the phenol–$H_2SO_4$ method[6]. The elution volumes were: 195 (dextran, mol.wt. 39,500), 330 (dextran, mol.wt. 10,000), 465 (dextran, mol.wt. 2,600), and 345 mL (aconitan A). The molecular weight was determined by comparison of the elution volume of aconitan A with the elution volumes of standard dextrans.

*Analysis of sugar components.* — Aconitan A was successively hydrolyzed, reduced, and acetylated[7]. The product was analyzed[8] by g.l.c. under conditions *A*: using a column (0.3 i.d. × 200 cm, spiral glass) packed with 3% OV-225 on Gaschrom Q (100–120 mesh) at 220° with a helium flow of 50 mL/min. The content of glucose determined by the chromotropic acid method[9], was 109.9%.

*Methylation analysis.* — NaH (25 mg) was mixed with $(CH_3)_2SO$ (5 mL) by ultrasonication for 30 min followed by stirring for 1 h at 70° and added to aconitan A (10.3 mg) in $(CH_3)_2SO$ (2 mL). After stirring for 4 h at room temperature, MeI (4 mL) was added to the mixture, which was stirred overnight at room temperature. All procedures were performed under $N_2$. Water (20 mL) was added and the mixture was extracted 5 times with $CHCl_3$ (20 mL each). The combined extract was washed 5 times with water (100 mL each), dried ($Na_2SO_4$), and evaporated. The residue was methylated 3 further times under the same conditions. The final residue was dissolved in 2:1 $CHCl_3$–MeOH and applied to a column (1.5 i.d. × 25 cm) of Sephadex LH-20. The column was eluted with the same solvent, and 3-mL fractions were collected. The eluates obtained from tubes 7–11 were combined and evaporated to yield the final product (15 mg), which showed no hydroxyl absorption in its i.r. spectrum.

The product (2 mg) was dissolved in 5M $H_2SO_4$–AcOH (1:19, 0.5 mL) and heated for 16 h at 80°, and then 0.25M $H_2SO_4$ (0.5 mL) was added to the solution, which was heated for 3 h at 100°. After being made neutral with Dowex-2 ($OH^-$), the filtrate and the washings with MeOH were combined and evaporated. The residue was dissolved in water (2 mL) in an ultrasonic bath for 5 sec and reduced with $NaBH_4$ (10 mg) for 18 h at 5°. After adjustment with Dowex 50W-X8 ($H^+$) to pH 5.0, the filtrate was evaporated and $H_3BO_3$ was removed by repeated addition and evaporation of MeOH. The product was acetylated with 1:1 $Ac_2O$–$C_5H_5N$ (2 mL) for 1 h at 100°. The solution was evaporated and the residue was dissolved in 1:1 $CHCl_3$–MeOH, and subjected to g.l.c.–m.s. on the same column as conditions *A* at 200° with a He flow of 60 mL/min. The relative retention-times of the products with respect to 1,5-di-*O*-acetyl-2,3,4,6-tetra-*O*-methyl-D-glucitol, and the main fragments in the mass spectra, are listed in Table I.

*Periodate oxidation followed by Smith degradation.* — Aconitan A (10.8 mg) was dissolved in water (2.5 mL) to which 0.1M $NaIO_4$ (2.5 mL) had been added. The mixture was kept at 5° in the dark. The periodate consumption was measured spectrophotometrically[10,11]. After 5 days, the formic acid liberated was determined by titration with 0.01M NaOH after the addition of ethylene glycol.

The residual mixture was treated with ethylene glycol (0.04 mL) for 1 h at 3°,

TABLE I

METHYLATION ANALYSIS OF ACONITAN A

| *Methylated sugar*[a] | T[b] | *Mol %* | *Mode of linkage* |
|---|---|---|---|
| 2,3,4,6-Glc | 1.00 | 1.2 | (terminal)-Glc*p*-(1→ |
| 2,3,4-Glc | 2.05 | 9.0 | →6)-Glc*p*-(1→ |
| 2,4-Glc | 3.60 | 1.0 | →3,6)-Glc*p*-(1→ |

[a]2,3,4,6-Glc = 2,3,4,6-tetra-*O*-methyl glucose, *etc.* [b]Retention time of the corresponding alditol acetate, relative to 1,5-di-*O*-acetyl-2,3,4,6-tetra-*O*-methyl-D-glucitol, on a column of OV-225 at 200° with He flow of 60 mL/min.

and $NaBH_4$ (45 mg) was added. After 16 h at 3°, the solution was adjusted to pH 5.0 by the addition of AcOH and dialyzed against water. The non-dialyzable fraction was concentrated and applied to a column (2.6 i.d. × 91 cm) of Sephadex G-15, which was eluted with water, collecting 20-mL fractions. The eluates obtained from tubes 9–11 were combined and lyophilized. The product was hydrolyzed with 0.5M $H_2SO_4$, containing D-mannitol as the internal standard, for 6 h at 100°. After being made neutral with Dowex-2 ($OH^-$), the hydrolyzate was reduced and acetylated as already described, and the resulting alditol acetates were analyzed by g.l.c. under conditions *A*.

*Acetolysis*. — Aconitan A (10 mg) was suspended in a 24:16:3 mixture (0.5 mL) of $Ac_2O$–AcOH–conc. $H_2SO_4$ and kept for 112 h at 30°. A part of the mixture (0.1 mL) was poured into water (1 mL) and extracted 3 times with 1-mL portions of $CHCl_3$. The combined extract was washed 3 times with 1-mL portions of water and 3 times with 1-mL portions of saturated $NaHCO_3$. After washing 5 times with water, the $CHCl_3$ extract was dried ($Na_2SO_4$) and evaporated. The residue was dissolved in MeOH (0.05 mL) to which 0.1M NaOMe (0.05 mL) had been added. After 3 h at room temperature, the mixture was diluted with water (1 mL) and made neutral with Dowex 50W-X8 ($H^+$). The filtrate was evaporated and subjected to t.l.c. on a precoated Kieselgel 60 (Merck) plate developed with 2:1:1 BuOH–AcOH–water. Zones were detected by spraying with 0.2% orcinol in 20% $H_2SO_4$, followed by heating for 5 min at 110°. The $R_F$ values of glucose and nigerose were 0.55 and 0.44, respectively.

The residue of the sample for t.l.c. was dried, trimethylsilylated conventionally[12], and subjected to g.l.c. on a column (0.3 i.d. × 200 cm, spiral glass) packed with 2% OV-101 on Uniport HP (80–100 mesh) and with a programmed temperature-increase of 4°/min from 180 to 300° at a He flowrate of 50 mL/min. Retention times (min) of $Me_3Si$-Glc were 9.7 and 11.4, and those of $Me_3Si$-nigerose were 26.7 and 27.1. One third of a standard sample of nigerose and all of a standard sample of isomaltose were converted into glucose under the acetolysis conditions already described.

## REFERENCES


1 C. Konno, M Murayama, K. Sugiyama, M. Arai, M Murakami, M Takahashi and H. Hikino, *Planta Med.*, (1985) 160.
2 S. Hakomori, *J. Biochem (Tokyo)*, 55 (1964) 205–208.
3 H. Bjorndal, B. Lindberg, and S. Svensson, *Carbohydr. Res.*, 5 (1967) 433–440.
4 M. Abdel-Akher, J. K Hamilton, R Montgomery, and F. Smith, *J. Am. Chem. Soc.*, 74 (1952) 4970–4971
5 K. Matsuda, H. Watanabe, K. Fujimoto, and K. Aso, *Nature (London)*, 191 (1961) 278.
6 M. Dubois, K. A. Gilles, J K. Hamilton, P A. Rebers and F Smith, *Anal. Chem.*, 28 (1956) 350–356.
7 M Tomoda, N. Satoh, and C. Ohmori, *Chem. Pharm. Bull.*, 26 (1978) 2768–2773.
8 M. Tomoda, N. Shimizu, K. Shimada, R Gonda and H Sakabe, *Chem. Pharm. Bull.*, 32 (1984) 2182–2186.
9 B Klein and M. Weissman, *Anal. Chem.*, 25 (1953) 771–774
10 J S Dixon and D. Lipkin, *Anal Chem*, 26 (1954) 1092–1093.
11 G. O. Aspinall and R. J. Ferrier, *Chem. Ind. (London)*, (1957) 1216–1220.
12 C. C. Sweeley, R Bentley, M. Makita, and W. W. Wells, *J. Am. Chem. Soc.*, 85 (1963) 2497–2507.