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# A NEW ACYLATED *N*-GLYCOSYL LACTAM FROM *ARISTOLOCHIA CONTORTA*[1]

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ABSTRACT.—A new acylated glycoside isolated from the roots of *Aristolochia contorta* (Aristolochiaceae), has been characterized as aristolactam *N*-(6′-*trans*-*p*-coumaroyl)-β-D-glucopyranoside [**2**]. Aristolactam-*N*-β-D-glucopyanoside [**1**] was also isolated from the same source. Compound **2** showed relatively significant antibacterial activity against Gram-positive bacteria, based on disc diffusion and dilution methods.

In the previous papers (1,2), we reported the isolation and structure identification of several components from the roots of *Aristolochia contorta* Bunge (Aristolochiaceae). As a continuation of our phytochemical studies on the constituents of this plant, we have isolated the known compound **1** and a new acylated *N*-glycosyl lactam characterized as **2**. The inhibitory effect of these compounds on bacteria was also tested.

## RESULTS AND DISCUSSION

Compounds **1** and **2** showed their general spectral features similar to those for aristolactam I (3). The ir spectra showed intense absorption bands at about 1030 and 1080 $cm^{-1}$ for glycosidic bonds. In the $^1$H-nmr spectra (Table 1), the coupling constants ($J = 9.3$ Hz) of the anomeric protons indicated the β-anomers for both **1** and **2**. The splitting patterns and the chemical shifts of two doublets, two singlets, and one triplet

TABLE 1. $^1$H-nmr Data of Compounds **1–4**.

| Proton | Compound | | | |
|---|---|---|---|---|
| | **1**[a] | **2**[a] | **3**[b] | **4**[b] |
| H-2 | 7.76 (s) | 7.76 (s) | 7.76 (s) | 7.83 (s) |
| H-5 | 8.23 (d) $J = 8.1$ | 8.17 (d) $J = 7.9$ | 8.19 (d) $J = 8.0$ | 8.25 (d) $J = 8.0$ |
| H-6 | 7.58 (t) $J = 8.1$ | 7.55 (t) $J = 8.0$ | 7.43 (t) $J = 8.2$ | 7.48 (t) $J = 8.0$ |
| H-7 | 7.22 (d) $J = 8.1$ | 7.21 (d) $J = 8.1$ | 7.00 (d) $J = 8.1$ | 7.01 (d) $J = 7.4$ |
| H-9 | 7.67 (s) | 7.67 (s) | 7.53 (s) | 7.60 (s) |
| H (anomeric) | 5.37 (d) $J = 9.3$ | 5.46 (d) $J = 9.3$ | 5.83 (d) $J = 9.5$ | 5.93 (d) $J = 9.2$ |
| -$OCH_2O$- | 6.51 (d) $J = 4.3$ | 6.50 (d) $J = 4.4$ | 6.30 (s) | 6.36 (s) |
| OMe | 4.03 (s) | 4.00 (s) | 4.00 (s) | 3.94 (s) |
| H-3″, -5″ | | 6.73 (d) $J = 8.2$ | | 7.09 (d) $J = 8.0$ |
| H-2″, -6″ | | 7.50 (d) $J = 8.2$ | | 7.50 (d) $J = 8.0$ |
| H-7″ | | 7.52 (d) $J = 15.8$ | | 7.67 (d) $J = 16$ |
| H-8″ | | 6.43 (d) $J = 15.8$ | | 6.41 (d) $J = 16$ |
| OAc | | | 1.74 | 1.80 |
| | | | 1.97 | 2.04 |
| | | | 2.06 | 2.12 |
| | | | 2.09 | 2.30 |

[a]In DMSO-$d_6$ (300 MHz).
[b]In $CDCl_3$ (80 MHz).

[1]In memory of Dr. B.S. Chung, 1929–1989.

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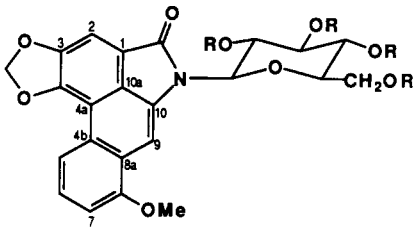
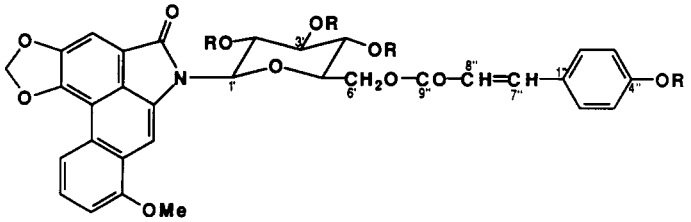


**1** R=H
**3** R=Ac



**2** R=H
**4** R=Ac

in the aromatic regions of **1** and **2** suggested that these two compounds have similar features in their molecular structures. An intensive mass spectral fragment *m/z* 293 was also present in the mass spectrum of aristolactam I, the aglycone of these two glycosides.

The close similarity of the $^1$H-nmr spectra of the two glycosides and aristolactam I and their co-occurrence in the same plant prompted us to re-examine the structures of the two compounds by $^{13}$C nmr using SFORD and DEPT methods. The anomeric configuration of the glycosidic linkages in **1** and **2** was determined by the following optical rotational and $^{13}$C-nmr spectral studies. The specific rotations $[\alpha]^{20}$D were $-16.8°$ and $-20.0°$ for **1** and **2**, respectively. These values were close to that of 9-β-D-glucopyranosyl adenine, $-14°$ (4). These data indicated that the sugar moiety was in the D form. The glucose fragment was identified by gc analysis of the TMSi derivative of **1**.

In the $^{13}$C-nmr spectrum of **1**, the glycosidation shift values (5) $\Delta\delta_A$ of the aglycone moiety were $\Delta\delta_A$ $C_\beta$ (C=O)$=-2.3$ ppm, $\Delta\delta_A$ $C_\beta$ ($C_{10}$)$=-1.2$ ppm, and $\Delta\delta_A$ $C_\tau$ ($C_9$)$=2.5$ ppm, and the residual part was similar to aristolactam I. ($^{13}$C-nmr chemical shifts of aristolactum I are to be reported in *Korean J. Pharmacogn.*, in press. $^{13}$C-nmr chemical shifts of C=O, $C_9$ and $C_{10}$ in aristolactam I were 168.47, 97.73, and 134.69, respectively.) As for the sugar moiety, the glycosidation shift value $\Delta\delta_S$ of the anomeric carbon $\Delta\delta_S$(C-1′)$=-14$ ppm, and the anomeric carbon shift of 81.8 ppm suggested that the glucose moiety was directly linked to the nitrogen atom of the aglycone (6,7).

Based on these lines of accumulated evidence, the structure for **1** was tentatively defined as aristolactam *N*-β-D-glucopyranoside, and $^{13}$C-nmr chemical shifts could be assigned (Table 2). This glycoside had previously been isolated from *Aristolochia indica* (8) as merely the β-D-glucoside of aristolactam I, but our assigned $^{13}$C-nmr data determined that this glucoside is a glucopyranoside.

In the $^1$H-nmr spectra of **2**, two proton signals (δA=6.43, δB=7.52, $J$=15.8) in an AB pattern suggested the presence of a trans-olefinic bond, while four protons (δ 6.73 and δ 7.50, $J$=8.2) could be attributed to the aromatic hydrogens in a para-disubstituted aromatic ring. This finding, along with the remaining carbon shifts of **2** in

TABLE 2. $^{13}$C-nmr Data of Compounds **1** and **2**.

| Carbon | Compound | |
|---|---|---|
| | **1**[a] | **2**[b] |
| C-1 | 124.24 (C)[c] | 124.20 (C)[c] |
| C-2 | 105.85 (CH) | 105.94 (CH) |
| C-3 | 148.79 (C) | 148.78 (C) |
| C-4 | 147.53 (C) | 147.55 (C) |
| C-4a | 110.99 (C) | 110.95 (C) |
| C-4b | 124.85 (C)[c] | 124.82 (C)[c] |
| C-5 | 118.61 (CH) | 118.59 (CH) |
| C-6 | 126.31 (CH) | 126.34 (CH) |
| C-7 | 108.51 (CH) | 108.41 (CH) |
| C-8 | 155.39 (C) | 155.30 (C) |
| C-8a | 117.61 (C) | 117.48 (C) |
| C-9 | 100.19 (CH) | 110.14 (CH) |
| C-10 | 133.52 (C) | 133.28 (C) |
| C-10a | 123.57 (C)[c] | 123.43 (C)[c] |
| -C=O | 166.19 (C) | 166.09 (C) |
| $-OCH_2O-$ | 103.34 ($CH_2$) | 103.38 ($CH_2$) |
| -OMe | 55.96 (Me) | 55.91 (Me) |
| C-1′ | 81.83 (CH) | 81.70 (CH) |
| C-2′ | 70.35 (CH)[d] | 69.93 (CH)[d] |
| C-3′ | 77.39 (CH) | 76.87 (CH)[e] |
| C-4′ | 69.83 (CH)[d] | 69.73 (CH)[d] |
| C-5′ | 80.02 (CH) | 76.37 (CH)[e] |
| C-6′ | 61.25 ($CH_2$) | 63.49 ($CH_2$) |
| C-1″ | | 124.93 (C) |
| C-2″ | | 130.19 (CH) |
| C-3″ | | 115.60 (CH) |
| C-4″ | | 159.71 (C) |
| C-5″ | | 115.60 (CH) |
| C-6″ | | 130.19 (CH) |
| C-7″ | | 144.93 (CH) |
| C-8″ | | 113.72 (CH) |
| C-9″ | | 166.51 (C) |

[a]In DMSO-$d_6$ (75 MHz).
[b]In DMSO-$d_6$ (100 MHz).
[c–e]Assignments may be reversed in each column.

comparison with **1**, is in full agreement with a *trans-p*-coumaric acid (9) as part of the structure of **2**. Indeed, treatment of **2** with methanolic KOH yielded **1** and *trans-p*-coumaric acid, and this result was further confirmed by hplc with internal standards.

In the $^{13}$C-nmr spectra of compound **2**, the carbon shift for the C-6′ signal has moved downfield by 2.2 ppm vs. **1** and that of the adjacent C-5′ upfield by 3.2 ppm, thus defining the acylation site as glucose C-6′, and this result was in line with other acylated glycosides (10–12).

Based on these results, the structure of the new acylated glycoside **2** was assigned as aristolactam *N*-(6′-*trans-p*-coumaroyl)-β-D-glucopyranoside.

Because aristolochic acids (1), 4,5-dioxoaporphine (2), 7-oxoaporphine (2), aristolactams, and aristolactam *N*-glycosides have been concurrently isolated from the same source, *A. contorta*, these components may be regarded as biogenetic intermediates in the biosynthetic pathway of aristolochic acids. Also, Castedo *et al.* (13) suggested that

aporphine alkaloids be postulated as precursors of aristolatams in plants. The biosynthetic pathway can be enlarged with the introduction of the oxoaporphinoids and aristolactams as possible intermediates of aristolochic acids.

## EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Melting points were determined on a Mitamura Ricken Heat Block Model and are uncorrected. All ir spectra were recorded in KBr pellets on a Beckman IR-20A spectrometer. The uv and $[\alpha]^{20}D$ were recorded in MeOH on an LKB ultraspec 4050 spectrometer and a JASCO DIPO-360 Digital Polarimeter, respectively. The nmr spectra were obtained in DMSO-$d_6$ using TMS as an internal standard on a Brucker AM 300 and a Brucker AM 400 NMR spectrometer. The ms spectra were recorded by a Hewlett Packard Model HP 5985 B and gc spectra by a Shimadzu G.C. 4BM. The hplc was carried out using a Bio-Rad HPLC with a uv detector under the reported conditions.

PLANT MATERIAL.—The roots of *A. contorta* were collected from Kyung-gi Do and Kang-won Do in Korea. The plant identification was verified by Dr. B.S. Chung, Seoul National University, where a specimen has been deposited.

EXTRACTION AND ISOLATION.—Air-dried roots (7 kg) were extracted with hot MeOH, and the solvent was removed in vacuo. The MeOH extract was successively fractionated with hexane, $CHCl_3$, and *n*-BuOH. The *n*-BuOH extract was chromatographed on Si gel with solvent $CHCl_3$-MeOH-$H_2O$ (100:14:1) and afforded 85 mg of **1** and 45 mg of **2**.

*Compound* **1**.—Aristolactam *N*-β-D-glucopyranoside ($C_{23}H_{21}O_9N$): ms *m/z* (rel. int. %) 293 (100.0) 278 (32.9), 263 (25.3); $[\alpha]^{20}D$ −16.8 ($c=3.6\times10^{-2}$% in MeOH); uv λ max (MeOH), 214, 242, 260, 290, 330, 396 nm; ir ν max $cm^{-1}$, 3400 (-OH), 2920 (-CH), 1680 (C=O), 1620 (C=C), 1080, 1030 (C-O-C); $^1H$ nmr see Table 1; $^{13}C$ nmr see Table 2.

HYDROLYSIS OF **1** AFTER $LiAlH_4$ REDUCTION.—Compound **1** (10 mg) and 30 mg of $LiAlH_4$ in THF were heated for 12 h in a three-necked flask. Workup and purification by tlc afforded the sample to be hydrolyzed. This reaction mixture was evaporated in vacuo and hydrolyzed with 10% HCl. The hydrolysate was neutralized and extracted with EtOAc. The residual $H_2O$ layer containing the sugar component was evaporated in vacuo and yielded the TMSi ether with hexamethyldisilazane (HMDS) and trimethylchlorosilane (TMCS). This TMSi ether showed the same retention time as that of glucose using the following gc conditions: column OV-1; detector fid; injection temperature 260° c; fid temperature 160° c; chart speed 0.5 cm/min; flow rate 1.0 ml/min; sensitivity $1\times10^2$ afs.

ACIDIC HYDROLYSIS OF **1** BY $HCO_2H$.—Compound **1** (10 mg) was hydrolyzed with 85% $HCO_2H$ solution in an oil bath at 175° c. The reaction mixture was treated by the same procedure as the LiAlH reduction product and afforded the TMSi ether. This TMSi ether showed the same retention time as that of glucose in the gc chromatogram using the same conditions for the hydrolysate of LiAlH reduction product.

ACETYLATION OF **1**.—Reaction of **1** (10 mg) with $Ac_2O$/pyridine gave the tetraacetate **3** (12 mg) as a slightly yellow crystalline powder: $C_{31}H_{29}O_{13}N$; ms *m/z* (rel. int. %), $[M]^+$ 623, [M−tetraacetyl glucose]$^+$ 293 (100.0), [293−OMe]$^+$ 263 (52.9); ir ν $cm^{-1}$ 3400, 2920, 1755, 1700, 1650, 1235, 1040; $^1H$ nmr see Table 1.

*Compound* **2**.—Yellow needles: mp 260–264°: $C_{32}H_2$-$O_{11}N$ (calcd C 64.17%, H 4.49%, N 2.25%, found: C 63.3%, H 4.97%, N 2.33%); sims (DMSO+glycerin) *m/z* (rel. int. %) $[M+H]^+$ 602 (25.98), [M−H−CO−CH=CH·$C_6H_4OH$]$^+$ 456 (14.51), [M−H−CO·CH=CH·$C_6H_3OH$·$C_6H_{10}O_5$]$^+$ 294 (4.8); $[\alpha]^{20}D$ −20.2 ($c=4.3\times10^{-2}$ % in MeOH); uv λ max (MeOH) 208, 235, 295, 390 nm; ir ν max $cm^{-1}$ 3430 (-OH), 2930 (-CH), 1690, 1650 (conj·C=O), 1615, 1085, 1025 (C-O-C); $^1H$ nmr see Table 1; $^{13}C$ nmr see Table 2.

DEACETYLATION OF **2** BY ALKALINE HYDROLYSIS.—Compound **2** (10 mg) solubilized in MeOH was added to 2 ml of 0.7M$K_2CO_3$ and left overnight at room temperature. The reaction mixture was evaporated in vacuo and identified as **1** and *trans-p*-coumaric acid which was further identified by hplc with internal standards using the following conditions: column Lichrosorb RP-18; uv detector 254 nm; flow rate 1.0 ml/min; aufs 0.02; chart speed 0.5 cm/min, mobile phase MeOH-$H_2O$ (2:8) (for *trans-p*-coumaric acid), MeOH-$H_2O$ (6:4) (for compound **1**).

ACETYLATION OF **2**.—Reaction of **2** (15 mg) with $Ac_2O$/pyridine gave a tetraacetate **4** (17 mg), slightly yellow needles: $C_{40}H_{35}O_{15}N$; ms *m/z* (rel. int. %) $[M]^+$ 769 (2.9), [M−acetyl coumarate]$^+$ 581 (4.6), [M−acetylcoumarate−OMe]$^+$ 551 (2.4), [acetyl coumarate]$^+$ 189 (21.7); $^1H$ nmr see Table 1.

ANTIBACTERIAL ACTIVITY.—The antibacterial activities of compounds **1** and **2** were tested using disc diffusion and dilution methods (14) against *Bacillus subtilis, Staphyococcus aureus,* and *Sarcina lutea*. The MIC values for **2** against *B. subtilis* and *Sa. lutea* were 43.8 μg/ml and 175 μg/ml, respectively. Positive control experiments with chloramphenicol against *Sa. lutea* showed an MIC of 5 μg/ml under the same experimental conditions.

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