Optically Active *N*-Acetyldopamine Dimer of the Crude Drug "Zentai," the Cast-off Shell of the Cicada, *Cryptotympana* sp.

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Two optically active *N*-acetyldopamine dimers together with four phenolic monomers were isolated from the crude drug "Zentai," a cast-off shell of the cicada of *Cryptotympana* sp. (Cicadidae). The former two were 2-(3′,4′-dihydroxyphenyl)-1,4-benzodioxane derivatives carrying substituents at the 3 and 6 (or 7) positions, which are known to be components of sclerotized insect cuticles. Their structures including absolute configurations were determined on the basis of NMR and circular dichroism (CD) spectroscopic data.

Key words *Cryptotympana* sp.; *N*-acetyldopamine dimer; 1,4-benzodioxane; cicada; cuticle; Cicadidae

The traditional Chinese crude drug, "Zentai," is a cast-off shell of the cicada of *Cryptotympana* sp. or *Platylomia* sp. (Cicadidae). This drug has been used as an antifebrile, a spasmolytic and an antiphlogistic. In addition, this was found to exhibit a setative and sympathetic ganglionic blocking activities.[1] But there is no report of components, except for the presence of chitin and amino acids.[1]

We examined the constituents of this drug, and isolated two 1,4-benzodioxane derivatives corresponding to *N*-acetyldopamine dimers (**5**, **6**) together with four phenolic monomers (**1**—**4**). The former two are known as the components of sclerotized insect cuticles, and they have already been obtained from the femurs of locusts and beetles by Anderson and Roepstorff.[2] They did not report, however, whether these compounds were optical active, and therefore, the absolute configuration as well as determination of the positional isomers remains unclear. Quite recently, Fujita and co-workers have also isolated four optically inactive 1,4-benzodioxane analogues from the cockroach belonging to Epilampridae.[3]

Because two 1,4-benzodioxane derivatives isolated in this study are optical active, we determined their structures including the absolute configurations. The structures of other phenolic monomers were also characterized.

Isolation of Compounds 1—6 The MeOH extract of a cast-off shell of the cicada was treated with ether to give ether-soluble and insoluble portions. The latter gave, on concentration, a fraction, which was shaken with *n*-BuOH–AcOEt and H_2O. As described in the experimental section, the *n*-BuOH–AcOEt soluble fraction was subjected to HPLC using a recycling mode to afford compounds, **1**—**6**.

Structures of Phenolic Monomers 1—4 Compounds **1** and **2** were identified respectively as 3,4-dihydroxybenzaldehyde and 3,4-dihydroxybenzoic acid by comparison of their spectral data with those of authentic samples.

Compound **3** exhibited the molecular ion peak at *m*/*z* 195, while compound **4** showed the molecular ion peak at *m*/*z* 209, in their electron impact mass spectra (EI-MS). Their ^{1}H- and ^{13}C-NMR spectroscopic analyses revealed that **3** and **4** are *N*-acetyldopamine and 2-oxo-*N*-acetyldopamine, respectively (Fig. 1).

Structures of *N*-Acetyldopamine Dimers, 5 and 6 The ^{1}H-NMR spectrum of **5** (FAB-MS *m*/*z*: 387 $[M+H]^+$, $[\alpha]_D$ +51.7°) showed two ABC-type signals in the aromatic region (δ 6.5—7.0), and signals ascribable to two methylenes (δ 2.63 and 3.26) and two methines (δ 4.75, d, J=7.3 Hz and δ 5.61, dd, J=7.3, 9.3 Hz) together with four signals due to OH and/or NH protons. Its ^{13}C-NMR spectrum exhibited twelve signals in the aromatic region in addition to two signals each of methine, methylene and carbonyl carbons. These observations together with the ^{1}H–^{1}H and ^{1}H–^{13}C chemical shift correlation spectroscopy (COSY) and two dimensional nuclear Overhauser spectroscopy (NOESY) spectral data revealed that **5** consists of unit A (3,4-disubstituted phenyl group linked to a *N*-acetylamino-2-ethyl group) and unit B (dihydroxyphenyl group and two oxymethine carbons) (Fig. 2). The ^{1}H-detected heteronuclear multiple bond connectivity (HMBC) spectrum of **5** showed clear correlation peaks as depicted in figure 2; however, no significant correlation between the A and B units was observed.

Treatment of **5** with diazomethane gave two compounds, **5a** and **5b**, in the ratio of 8 : 1. In their positive ion FAB-MS, both of them exhibited the *quasi*-molecular ion peak at *m*/*z* 415, which was 28 mass units more than that of **5**. Interestingly, unlike the starting material (**5**), both compounds showed specific rotation, $[\alpha]_D < \pm 0.05°$ (c=1.0, MeOH), and gave no CD maximum, indicating that they were racemates.

The ^{1}H-NMR spectra of **5a** and **5b** were quite similar to each other, but the methine proton signals of the former were observed at δ 4.84 (d, J=7.3 Hz) and δ 5.65 (dd, J=7.3,

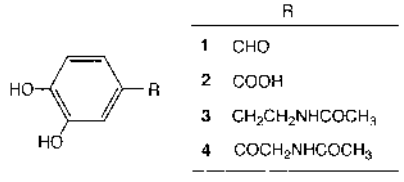

Fig. 1. Structures of **1**—**4**

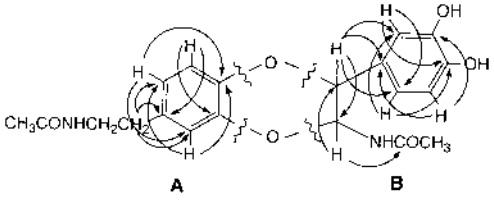

Fig. 2. HMBC Correlation

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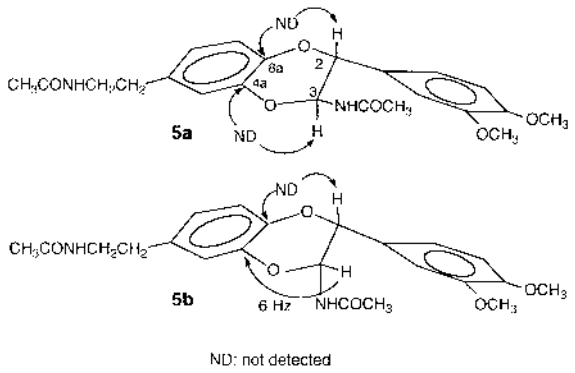

Fig. 3. HMBC Correlation of H-2/C-8a and H-3/C-4a of **5a** and **5b**

9.7 Hz), while those of the latter appeared at δ 5.22 (d, J=2.0 Hz) and δ 5.98 (dd, J=2.0, 10.1 Hz). These findings showed that they are racemates of 1,2-disubsutituted *cis* and *trans* stereoisomers in the dioxane ring, which were formed under the methylation condition.

The HMBC spectrum of **5a** gave, similar to that of **5**, no diagnostic cross peak between the A and B units; in contrast, that of **5b** showed a clear long-range correlation peak between H-3 (δ 5.98) and C-4a (δ 140.6) which connects A with B, thereby constructing a 1,4-benzodioxane framework.

The $^3J_{CH}$ coupling constants are related to the dihedral angle between carbon and proton, as known from the Karplus-Conroy relation for $^3J_{HH}$.[4] In view of the above HMBC spectral observations, it was proven that the H-2 and H-3 bonds in **5a**, and the H-2 bond in **5b** stand vertically on the 1,4-benzodioxane ring ($^3J_{CH}$<1 Hz), while the H-3 bond of **5b** is located almost in the 1,4-benzodioxane plane ($^3J_{CH}$=6 Hz)[5] (Fig. 3).

Detailed analyses of the HMBC and NOESY spectra of **5a** and **5b** demonstrated that the *N*-acetylamino-2-ethyl group is located at the 6 position, and the two methoxy groups are located at the 3′ and 4′ positions in the phenyl group. Based on the coupling constant ($J_{2H,3H}$=7.3 Hz) and the above HMBC spectral observations of **5a**, the stereochemistry of the 2 and 3 positions was concluded to be *trans* (H-2 axial, H-3 axial) form. On the other hand, compound **5b** has the *cis* (H-2 axial, H-3 equatorial) form, that is, the 3-epimer of **5a**.

As the parent compound **5** showed the same coupling constant ($J_{2H,3H}$=7.3 Hz) as that of **5a**, it was concluded that the 2-phenyl and 3-acetylamino groups of **5** have a 2,3-*trans* (H-2 axial, H-3 axial) configuration.

The ^{1}H- and ^{13}C-NMR spectra of **6** (*m*/*z* 387 $[M+H]^+$, $[\alpha]_D$ +28.2°) were quite similar to those of **5**, suggesting that **6** is a positional isomer of **5**.

Treatment of **6** with diazomethane gave two racemates, **6a** and **6b**, in the ratio of 8 : 1. The NMR spectral data including the HMBC correlations of **6a** and **6b** were almost identical with those of the respective **5a** and **5b**. The HMBC and NOESY spectral analyses of **6a** and **6b** revealed that they differ only in the position of the substituted group, the *N*-acetylamino-2-ethyl group, placed at the 7-position instead of the 6-position in **5a** and **5b**.

Determination of the Absolute Configuration In 1985, Arnoldi and Merlini obtained (2*R*,3*S*)- and (2*S*,3*S*)-3-methyl-2-phenyl-1,4-benzodioxanes by stereospecific synthesis, and their absolute stereochemistry was determined by the CD

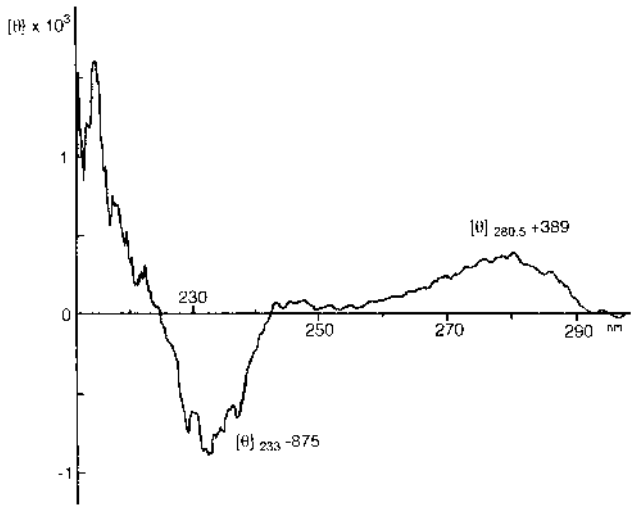

Fig. 4. CD Spectrum of **5**

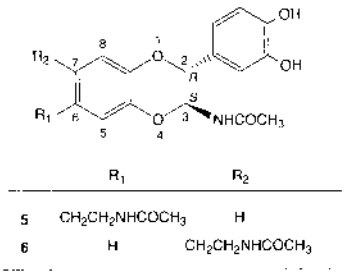

Fig. 5. Structures of **5** and **6**

spectral data.[6] In addition, Quaglia *et al.* synthesized other optically active 1,4-benzodioxane derivatives, and their CD curves were presented.[7] These reports showed that the CD curves of the 1,4-benzodioxanes having the phenyl group linked directly with the 2-upper equatorial position (2*S* form) exhibited a maximum at *ca.* 230 nm (a positive sign in the range of 225—240 nm), and a negative Cotton effect in the range of 270—290 nm. While those having the phenyl group attached at the 2-lower equatorial position (2*R* form) displayed almost the mirror image. The CD spectrum of **5** showed a negative sign in the range of 225—240 nm, and a positive sign in the range of 270—290 nm (Fig. 4), and this spectrum was quite similar to those of the 1,4-benzodioxanes having the phenyl group attached directly at the 2-lower equatorial position. From the results obtained above, it was clear that the 3′,4′-dihydroxyphenyl group in **5** was located at the 2-lower equatorial position, and thus the structure of **5** was defined as (2*R*,3*S*)-2-(3′,4′-dihydroxyphenyl)-3-acetylamino-6-(*N*-acetyl-2″-aminoethyl)-1,4-benzodioxane.

Compound **6** gave an almost identical CD curve as that of **5**, showing the same chiral center (2*R*,3*S*) as that of **5**, and hence **6** was assigned the structure (2*R*,3*S*)-2-(3′,4′-dihydroxyphenyl)-3-acetylamino-7-(*N*-acetyl-2″-aminoethyl)-1,4-benzodioxane.[8] Figure 5 depicts their structures.

To further confirm the above conclusion, we attempted to prepare a suitable derivative for X-ray crystallographic analysis; however, an optical active crystal could not be obtained due to racemization. The mechanism of easy racemization of these dimers is as yet unclear.

Two 1,4-benzodioxane derivatives isolated from animal

Table 1. ^{1}H-NMR Spectral Data (DMSO-d_6)

^{1}H	5	5a	5b	6	6a	6b
2	4.75 (d, 7.3)	4.84 (d, 7.3)	5.22 (d, 2.0)	4.72 (d, 7.3)	4.86 (d, 7.3)	5.24 (d, 2.0)
3	5.61 (dd, 7.3, 9.3)	5.65 (dd, 7.3, 9.7)	5.98 (dd, 2.0, 10.1)	5.56 (dd, 7.3, 9.5)	5.64 (dd, 7.3, 9.7)	5.97 (dd, 2.0, 10.1)
5	6.76 (d, 2.0)	6.77 (d, 2.0)	6.78 (d, 2.0)	6.72 (d, 8.6)	6.84 (d, 8.1)	6.84 (d, 8.1)
6	—	—	—	6.68 (dd, 2.0, 8.6)	6.73 (dd, 2.0, 8.1)	6.77 (dd, 2.0, 8.1)
7	6.70 (dd, 2.0, 8.0)	6.71 (dd, 2.0, 8.3)	6.75 (dd, 2.0, 8.1)	—	—	—
8	6.74 (d, 8.0)	6.88 (d, 8.3)	6.96 (d, 8.1)	6.71 (d, 2.0)	6.82 (d, 2.0)	6.90 (d, 2.0)
2′	6.81 (d, 2.0)	7.04 (d, 2.0)	7.23 (d, 2.0)	6.78 (d, 2.0)	7.05 (d, 2.0)	7.23 (d, 2.0)
5′	6.71 (d, 8.0)	6.96 (d, 8.1)	6.95 (d, 8.5)	6.78 (d, 8.4)	6.96 (d, 8.3)	6.96 (d, 8.5)
6′	6.86 (dd, 2.0, 8.0)	6.97 (dd, 2.0, 8.1)	7.07 (dd, 2.0, 8.5)	6.82 (dd, 2.0, 8.4)	6.98 (dd, 2.0, 8.3)	7.07 (dd, 2.0, 8.5)
1″	2.63 (t, 8.4)	2.61 (t, 7.4)	2.63 (t, 7.4)	2.60 (t, 8.5)	2.62 (t, 7.4)	2.64 (t, 7.4)
2″	3.26 (td, 5.2, 8.4)	3.22 (td, 5.4, 7.4)	3.22 (td, 5.4, 7.4)	3.21 (td, 5.2, 8.4)	3.23 (td, 5.4, 7.4)	3.23 (td, 5.4, 7.4)
CH_3	1.80 (s), 1.81 (s)	1.78 (s), 1.79 (s)	1.77 (s), 1.79 (s)	1.80 (s), 1.81 (s)	1.78 (s), 1.79 (s)	1.76 (s), 1.79 (s)
OCH_3	—	3.75 (s), 3.76 (s)	3.76 (s), 3.77 (s)	—	3.75 (s), 3.76 (s)	3.76 (s), 3.77 (s)

δ in ppm from TMS (splitting patterns and coupling constants); J in Hz are given in parentheses.

Table 2. ^{13}C-NMR Spectral Data (DMSO-d_6)

^{13}C	5	5a	5b	6[a)]	6a	6b
2	76.1	76.0	75.8	78.3	76.1	75.6
3	76.7	76.6	75.1	78.3	76.6	74.9
5	116.6	116.6	117.3	118.0	116.5	117.1
6	133.0	133.2	133.7	123.2	121.9	122.3
7	121.5	121.5	121.4	134.2	132.8	132.7
8	116.4	116.5	117.2	118.2	116.7	117.3
4a	141.9	141.0	140.6	142.2	140.5	139.1
8a	141.1	141.9	141.5	144.3	142.4	142.8
1′	126.9	128.3	128.0	128.9	128.3	127.9
2′	114.8	111.3	110.8	115.6	111.3	110.7
3′	145.0	148.4	148.5	146.5	148.4	148.4
4′	145.7	149.0	148.7	147.1	149.0	148.7
5′	115.2	111.4	111.3	116.2	111.4	111.2
6′	118.9	120.0	119.3	120.6	120.0	119.2
1″	34.4	34.3	34.4	35.8	34.4	34.4
2″	40.1	40.2	40.3	42.2	40.2	40.2
CH_3	22.5	22.5	22.3	22.6	22.5	22.2
	22.5	22.5	22.6	22.6	22.5	22.5
OCH_3	—	55.4	55.4	—	55.4	55.4
	—	55.5	55.6	—	55.5	55.6
CO	169.0	169.0	169.1	173.3	169.4	169.0
	169.0	169.4	170.3	173.3	169.4	170.2

a) Compound **6** was dissolved in CD_3OD. δ in ppm from TMS.

sources in the present study are, unlike those so far reported, the first examples of optically active dimers, which consist of 2 units of the *N*-acetyldopamine. Recently some 1,4-benzodioxanes with a high affinity for 5-HT receptor subtypes have been shown to have neuroleptic activity, suggesting that they are predictive tools for novel anxiolytic agents.[9)] This information leads us to suppose that the sedative activity of the drug, "Zentai," is probably due to the *N*-acetyldopamine dimers.

Experimental

The NMR spectra were recorded on a JEOL JNM GX-400 instrument at 400 MHz (^{1}H) and 100 MHz (^{13}C) at a probe temperature of 35 °C using tetramethylsilane (TMS) as an internal reference. The abbreviations used are as follows: s, singlet; brs, broad singlet; d, doublet; t, triplet; dd, double doublet; td, triple doublet; m, multiplet. MS were acquired on a JEOL JMS DX-300 spectrometer (EI-MS: ionization voltage, 30 eV; accelerating voltage 3—10 kV; positive ion FAB-MS: accelerating voltage 3 kV; matrix, glycerol; collision gas, Xe). CD spectra were recorded on a J-725 (JASCO) in MeOH (2 mM). Optical rotations were measured at 25 °C with a JASCO DIP-140 polarimeter. TLC was carried out on silica gel HPTLC with Al sheets (Merck Art. 5556). Spots were visualized with 5% H_2SO_4 in MeOH (by heating). Column chromatography was carried out on Merck Silica gel (230—400 mesh, Art. 9385). Preparative HPLC was conducted over Inertsil Prep ODS-3 and Inertsil Ph-3 (10 μm, 20×250 mm, GL Sciences) on a JASCO 880-PU equipped with a JASCO 830-RI. Recycling HPLC was carried out on a JASCO 880-PU equipped with a JASCO preparative recycle valve.

Isolation of Compounds 1—6 The crude drug (1 kg), "Zentai," a cast-off shell of the cicada of *Cryptotympana* sp. purchased from Tochimoto Tenkaido Co. was extracted with $CHCl_3$–MeOH (1 : 1, 5 l). The extract was concentrated to give a brown powder (18.4 g). This was treated with ether to give a dissolved fraction (1.1 g) and an insoluble fraction (15.2 g). The latter was shaken with $CHCl_3$–MeOH–H_2O (1 : 1 : 1, 900 ml) and the upper layer was collected. After removal of the solvent, the residue (4.6 g) was shaken with AcOEt–*n*-BuOH–H_2O (1 : 1 : 1, 300 ml) and the upper layer was collected. This was concentrated to give a fraction (fr. I, 3.4 g). Fr. I was subjected to preparative HPLC on an Inertsil Prep ODS-3 (size, 20×250 mm: 10 μm) using 20% CH_3CN as an eluent to give three fractions, fr. 1—fr. 3. Fraction 1 was further separated by HPLC on an Inertsil Ph-3 (size, 20×250 mm; 10 μm) using 18% CH_3CN as an eluent to give four fractions, fr. 4—fr. 7. Each fraction was subjected to HPLC (Inertial Ph-3) in a recycling mode using 12% CH_3CN as an eluent to give **1** (30 mg, from fr. 7), **2** (10 mg, from fr. 4), **3** (11 mg, from fr. 6) and **4** (19 mg, from fr. 5). Fraction 2 was subjected to HPLC (Inertsil ODS-3) in a recycling mode using 25% CH_3CN as an eluent to give **5** (83 mg) and **6** (68 mg). Compounds **1**—**2** were identified respectively as 3,4-dihydroxybenzaldehyde and 3,4-dihydroxybenzoic acid by comparison of their spectral data with those of authentic samples (Wako Pure Chemical Industries, Ltd.).

***N*-Acetyldopamine (3)** Yellow powder, mp 153—160 °C. EI-MS *m/z*: 195 $(M)^+$. ^{1}H-NMR (DMSO-d_6, 400 MHz) δ: 1.80 (3H, s, CH_3), 6.57 (d, J=2.0 Hz, H-2), 6.63 (d, J=8.3 Hz, H-5), 6.43 (dd, J=2.0, 8.3 Hz, H-6), 3.15 (2H, td, J=5.3, 7.3 Hz, H-1′), 2.50 (2H, t, J=7.3 Hz, H-2′), 7.86 (t, J=5.3 Hz, NH). ^{13}C-NMR (DMSO-d_6, 100 MHz) δ: 22.5 (CH_3), 34.6 (C-2′), 40.5 (C-1′), 115.3 (C-2), 115.8 (C-5), 119.0 (C-6), 130.1 (C-1), 143.4 (C-3), 144.9 (C-4), 168.9 (OAc).

2-Oxo-*N*-acetyldopamine (4) Yellow crystal, mp 168—175 °C. EI-MS *m/z*: 209 $(M)^+$. ^{1}H-NMR (DMSO-d_6, 400 MHz) δ: 1.91 (3H, s, CH_3), 7.35 (d, J=1.8 Hz, H-2), 6.83 (d, J=8.3 Hz, H-5), 7.38 (dd, J=1.8, 8.3 Hz, H-6), 4.46 (2H, d, J=5.2 Hz, H-2′), 7.94 (t, J=5.2 Hz, NH). ^{13}C-NMR (DMSO-d_6, 100 MHz) δ: 22.3 (CH_3), 45.1 (C-2′), 114.6 (C-2), 115.1 (C-5), 120.9 (C-6), 126.9 (C-1), 145.2 (C-3), 150.8 (C-4), 169.4 (OAc), 193.2 (C-1′).

(2*R*,3*S*)-2-(3′,4′-Dihydroxyphenyl)-3-acetylamino-6-(*N*-acetyl-2″-aminoethyl)-1,4-benzodioxane (5) Yellow powder, mp 118—126 °C. $[\alpha]_D$ +51.7° (c=1.0, MeOH). Positive ion FAB-MS *m/z*: 387 $(M+H)^+$. ^{1}H- and ^{13}C-NMR (DMSO-d_6, 400 MHz) δ: see Tables 1 and 2.

(2*R*,3*S*)-2-(3′,4′-Dihydroxyphenyl)-3-acetylamino-7-(*N*-acetyl-2″-aminoethyl)-1,4-benzodioxane (6) Yellow powder, mp 115—120 °C. $[\alpha]_D$ +28.2° (c=1.0, MeOH). CD $[\theta]_{280}$ +400, $[\theta]_{230}$ −905. Positive ion FAB-MS *m/z*: 387 $(M+H)^+$. ^{1}H- and ^{13}C-NMR (DMSO-d_6, 400 MHz) δ: see Tables 1 and 2.

Methylation of 5 and 6 Each compound (**5** and **6**, 50 mg each) was dissolved in MeOH (5 ml). Diazomethane in ether was added to this solution, until the spot due to the starting material disappeared by TLC. After removal of the solvent under a nitrogen stream, the residue was subjected to HPLC

on an Inertsil ODS-3 (size, 20×250 mm; 10 μm) using 33% CH_3CN as an eluent to give **5a** (36.1 mg) and **5b** (4.2 mg) from **5**, and **6a** (37.6 mg) and **6b** (4.8 mg) from **6**. NMR spectral data for these four compounds are shown in Tables 1 and 2.

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