

Synthesis of (\pm)-Gonyauline, an Endogenous Substance Shortening the Period of Circadian Rhythm in the Unicellular Alga *Gonyaulax polyedra*

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The synthesis of (\pm)-gonyauline **1**, *cis*-dimethylsulphoniocyclopropanecarboxylate, affecting the period of the circadian clock of the unicellular alga *Gonyaulax polyedra* is reported.

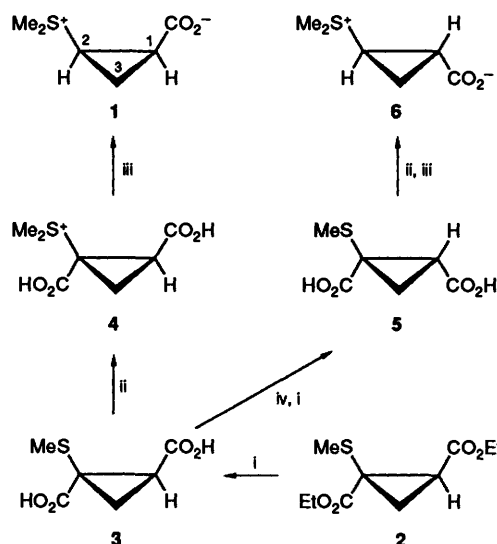
The cyclopropane compound gonyauline **1**,¹ containing a novel zwitterion structure composed of carboxylate and dimethylsulphonio functionalities, has recently been identified as an endogenous principle which shortens the period of circadian rhythm in the cultured marine unicellular alga *Gonyaulax polyedra*.² Gonyauline **1**, as well as creatine which has similar period-shortening effects,³ can be used to investigate the molecular mechanism of the cellular circadian clock. We describe here the synthesis of (\pm)-gonyauline, and establish the novel cyclopropane structure of this substance.

Alkaline hydrolysis (NaOH/MeOH-H₂O) of the readily available ethyl ester **2**⁴ gave the *trans*-dicarboxylic acid **3** (m.p. 147–148 °C, 50% yield after recrystallization)‡. Methylation of **3** with methyl toluene-*p*-sulphonate in formic acid⁶ yielded sulphonic acid salts of **4** (m.p. 153–154 °C, 44% yield). Decarboxylation was achieved by refluxing in *t*-butyl alcohol to give toluene-*p*-sulphonic acid salts of the *cis* isomer **1**. Purification on a DEAE-Sephadex ion exchange column followed by a Sephadex G-10 column afforded (\pm)-gonyauline **1** in 33% yield: hygroscopic needles; m.p. 141–143 °C.

On the other hand, *cis*-dicarboxylic acid **5**, obtained from **3** by inverting the stereochemistry at C-1 *via* the acid anhydride formation, yielded the *trans*-isomer of gonyauline **6** in 42% yield by the same sequence of reactions: hygroscopic amorphous solid; m.p. 146–151 °C; $\delta_{\text{H}}(\text{D}_2\text{O}; 500 \text{ MHz})$ 1.24 (Bu⁴OH), 1.56 (1 H, dt, J/Hz : 9, 6, 3-H), 1.64 (1 H, q, J 6 Hz, 3-H), 2.20 (1 H, ddd, J/Hz : 9, 6, 4, 1-H), 2.88 (1 H, td, J/Hz : 6, 4, 2-H) and 3.02 (6 H, s, Me₂S⁺); $\delta_{\text{C}}(\text{D}_2\text{O}; 125 \text{ MHz})$ 67.3 (dioxane), 13.6 (t, C-3), 23.9 (d, C-1), 27.1 (q, 2 × C, Me₂S⁺), 28.2 (d, C-2) and 176.7 (s, CO₂⁻). These results suggest that decarboxylation took place while retaining the configuration at C-1 position of **4**. In fact, one deuterium atom was regio- and stereo-specifically incorporated into C-2 position of **1** when the reaction was performed in Bu⁴OD.

These isomers **1** and **6** were easily distinguished from each other, and the *cis*-isomer **1** showed the same chromatographic properties (TLC, HPLC and column chromatography), mass spectral fragmentations, and ¹H (500 MHz) and ¹³C (125 MHz) NMR spectrum with those of the natural material,¹ which confirm the stereochemistry of natural gonyauline as shown in **1**. Furthermore, synthetic (\pm)-gonyauline exhibited a period-shortening effect on the circadian rhythm of luminescence of *Gonyaulax polyedra* similar to that of natural (+)-gonyauline.¹

Gonyauline might be derived biogenetically from methionine or its derivatives such as *S*-methylmethionine.⁵ It is noteworthy to mention that plant ageing hormone, ethylene, has been shown to be derived from methionine *via* an intermediate, 1-aminocyclopropanecarboxylic acid (ACC).⁶ Synthesis of



Scheme 1 Reagents and conditions: i, NaOH/MeOH-H₂O, r.t.; ii, TsOMe/HCOOH, 50 °C, 12 h; iii, Bu⁴OH, reflux, 24 h; iv, Ac₂O-AcOK, reflux, 1 h.

optically active gonyauline and studies on molecular mechanisms of period-shortening effect of gonyauline and creatine are in progress.

Experimental

(\pm)-*trans*-Dicarboxylic Acid **4**.—A solution of the diacid **3** (0.88 g, 5 mmol) in a mixture of methyl toluene-*p*-sulphonate (1.86 g, 10 mmol) and formic acid (8.8 cm³) was stirred at 50 °C for 10 h. The mixture was evaporated and the residue was dissolved in water. The solution was washed with ethyl acetate and evaporated to give colourless solids of toluene-*p*-sulphonic acid salts of **4** (800 mg, 44%): hygroscopic amorphous solids; m.p. 146–151 °C (Found: M⁺ - TsOH, 172.0209. C₇H₉O₃S requires 172.0226); $\nu_{\text{max}}(\text{KBr})/\text{cm}^{-1}$: 3430, 3040, 2550, 1730, 1410, 1220, 1155, 1145, 1125, 1035 and 1010; $\delta_{\text{H}}(\text{D}_2\text{O}; 500 \text{ MHz})$ 1.24 (Bu⁴OH), 2.01 (1 H, dd, J/Hz : 7.9, 6.2, 3-H), 2.23 (1 H, dd, J/Hz : 9.5, 6.2, 3-H), 2.37 (3 H, s, MeAr), 2.94 (1 H, dd, J/Hz : 9.5, 7.9, 2-H), 3.16 (3 H, s, MeS⁺), 3.18 (3 H, s, MeS⁺), 7.34 (2 H, d, J 8 Hz, Ar) and 7.69 (2 H, d, J 8 Hz, Ar); $\delta_{\text{C}}(\text{D}_2\text{O}; 125 \text{ MHz})$ 67.3 (dioxane), 21.1 (q, MeAr), 23.3 (t, C-3), 25.2 (q, MeS⁺), 25.5 (q, MeS⁺), 31.7 (d, C-2), 35.2 (s, C-1), 126.1 (d, Ar), 130.0 (d, Ar), 140.6 (s, Ar), 142.9 (C, Ar), 167.3 (s, CO₂H) and 170.7 (s, CO₂H).

(\pm)-Gonyauline **1**.—A solution of the toluene-*p*-sulphonic acid salts of **4** (257 mg, 0.71 mmol) in *t*-butanol was refluxed for 24 h. The mixture was evaporated and dissolved in water. The

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‡ All new compounds gave satisfactory spectral and analytical data.

water layer was washed with ethyl acetate, concentrated and chromatographed on a silica gel column [elution, BuOH–AcOH–H₂O (4:1:1)] followed by a DEAE-Sephadex column (elution, 0.02 mol dm⁻³ ammonium acetate in H₂O) and a Sephadex G-10 column (elution, H₂O) to yield (±)-gonyauline (34 mg, 33%); hygroscopic colourless needles (Found: M⁺, 146.0412. C₆H₁₀O₂S requires 146.0401); m.p. 141–143 °C; ν_{\max} (KBr)/cm⁻¹: 3420, 1590, 1410, 1315 and 1280; δ_{H} (D₂O; 500 MHz) 1.24 (Bu¹OH), 1.54 (1 H, q, *J* 6 Hz, 3-H), 1.69 (1 H, td, *J*/Hz 9, 6, 3-H), 2.26 (1 H, td, *J*/Hz 6, 9, 1-H), 2.78 (1 H, td, *J*/Hz 9, 6, 2-H), 2.90 (3 H, s, MeS⁺) and 3.00 (3 H, s, MeS⁺); δ_{C} (D₂O; 125 MHz) 14.6 (t, C-3), 23.4 (d, C-1), 27.6 (q, 2 × C, MeS⁺), 29.0 (d, C-2) and 177.0 (s, CO₂⁻).

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