Synthesis and Properties of Rhodamine-type Dyes Derived from Spiculisporic Acid

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Abstract

A new rhodamine-type fluorescent dye having a functional long alkyl moiety (xanthene dye) was synthesized and their properties were investigated. The anhydride of spiculisporic acid which was produced in the culture broth of Penicillium spiculisporum was reacted with m-diethylaminophenol to give rhodamine-type dyes. Aqueous solutions of the dye in the acidic conditions showed wine color $\lambda_{\text{max}}$ 571 nm, Fluorescence $\lambda_{\text{max}}$ 589 nm, differently from brilliant red colors in the conventional rhodamine dyes. Furthermore, hydrochloric acid salt of the dye was surface active (35.1 mN/m at $\gamma_{\text{cmc}}$) and formed micelles in water.

1. Introduction

It has been known that trace amounts of spiculisporic acid are contained as membranous constituents in microorganisms and lichens. From the recent completion of the high efficient production and the viewpoint of the utilization of amphipathic function of spiculisporic acid, present authors have studied on the applications for high-priced surfactants with safety and biodegradability[1,2]. In this report, a rhodamine-type dye was obtained from spiculisporic acid as a synthetic intermediate continuing to oligosoap-type fluorescent dyes[3].

2. Experimental

2.1 Materials

Spiculisporic acid (4,5-dicarboxy-4-pentadecanolide) was supplied from Iwata Chemical Co. Ltd. (Shizuoka), which was manufactured by the similar procedures of Tabuchi et al.4): namely, Penicilli um spiculisporum Lehman No 10-1 was incubated at 30°C for 4 days using a reciprocal shaker in glucose monohydrate (110 g), NH₄Cl (1.0 g), KH₂PO₄ (1.0 g), MgSO₄·7H₂O (0.5 g) and corn steep liquor (1 g) per liter of water, and spiculisporic acid was accumulated in a maximum yield of 110 culture broth under suitable cultural conditions. Spiculisporic acid anhydride was prepared by heating spiculisporic acid at 170~180°C in a reduced pressure of 1~2 mm torr. as shown in Fig. 1. The resulting anhydride was purified by repeating recrystallization from hexane-ether. Analytical results: spiculisporic acid (C₁₇H₂₈O₆); C, obsd. 62.22 (calcld. 62.17), H, 8.59 (8.59), O, 29.20 (29.23), IR, 1,715 cm⁻¹ (lactone), mp. 146°C, $[\alpha]_D^2 = -12.6$; Spiculisporic acid anhydride (C₁₇H₂₆O₅); C, 65.78 (65.83), H, 8.44 (8.41), IR, 1,800 and 1,780 (acid anhydride), 1,710 (COOH), 1,685 cm⁻¹ (double bond), mp. 41°C. m-Diethylaminophenol (99.1% purity) was supplied from Daiwa Dyestuff Manufacturing Co. Ltd.. Other chemicals were reagent grade ones.
2.2 Methods
Shimadzu fluorescence spectrophotometer was used to determine absorption and fluorescence spectra at a room temperature of about 25°C. Surface tension measurements were carried out by means of a Wilhelmy-type surface tensionmeter, Shimadzu ST-1 at 30±0.1°C.

3. Results and Discussion

3.1 Synthesis of a rhodamine-type dye
Spiculisporic acid anhydride, 3.1 g (0.01 mol) was reacted with m-diethylaminophenol, 3.3 g (0.02 mol) in a separable flask at 140-145°C for 0.5 hour under stirring. The resulting mixtures were cooled to 40°C and poured into 5% aqueous sodium hydroxide solutions to give dye base (IV) as precipitates. The precipitates were repeatedly washed with water and were dried in vacuo. The reaction pathways are shown in Fig. 1. The precipitates were analysed by means of thin layer chromatography (TLC) using the test plates of Wako silicagel 70(A) and the developing solvent of butanol : water (10 : 1). The precipitates were separated on the chromatogram; Rf value, 0.41 (small pink spot), 0.72 (main reddish violet one), 0.96 (brown one, unreacted m-diethylaminophenol). Then, the precipitates were purified by means of column chromatography using Wako silica gel C-200; namely, methanol solutions of the precipitates were poured into the column and were eluted with benzene as the developing solvent. The major fraction was identical with the chemical structure of IV from the elemental analysis, IR and NMR. The yield was about 5%. Analytical results of IV (C_{37}H_{45}N_{2}O_{5}) : elemental analysis, C, obsd. 73.09% (calcd. 73.48), H, 8.24 (8.67), N, 5.97 (4.48 ; IR, 2,950 cm⁻¹ (s., methylene chain), 1,810 and 1,770 (s., lactone), 1,715 (m., COOH), 1,460 and 1,380 (m., alkyl chain), 1,620 and 820 (m., aromatic) ; NMR, δ_{1,24} (alkanoic methylene chain), δ_{0.89} (terminal methyl).

3.2 Fluorescent characteristics
The dye base (IV) was not soluble in water and soluble in methanol. The methanol solution of IV produced the color of reddish violet and absorbed λ_{max} at 551 nm (ε=11,500), while the solution turned into a wine color (λ_{max} : 571 nm ε=24,000) on the addition of drops of aqueous hydrochloric acid due to the occurrence of a conjugated double bond (V). The color of V was different from brilliant red color of conventional rhodamine dyes. Absorption and fluorescence spectra of V are shown in Fig. 3. On the other hand, dye base (IV) has both λ_{max} at 551 nm and fluorescence λ_{max} at 565 nm (Fig. 4). This would be attributed to the presence of the inner salt structure (IV') equilibrating with IV as illu-

Fig. 2. Synthetic scheme of a rhodamine-type dye (V) from spiculisporic acid anhydride (I)
3.3 Dyeing property
Dyeing properties of V were examined in aqueous acidic ethanol solutions under the general dyeing conditions. Nylon, wool and silk fibers were dyed in wine colors at 70-95°C. Cotton and poly vinyl acetal (vinylon) fibers did not dye under the similar conditions. The effect of the long alkyl chain of properties seemed to be expected.

3.4 Surface activity
Aqueous dye (V) solutions exerted the remarkable actions for surface tension lowering as shown in Fig. 6. Critical micelle concentration (cmc) was detectable as a break point in Fig. 6, and was determined as 0.055% (8.2×10^-4 mol/L). So, it was confirmed that V formed micelles in water. Furthermore, the equilibrium value of surface tension (γcmc) was 35.1 mN/m from the search of Fig. 6.

4. Conclusion
Spiculisporic acid derivative having a rhodamine dye structure was newly prepared. Such a surface-active fluorescent dye may be expected to have many applications as markers for biological and pH-sensitive purpose, owing to the absorption shifts dependent on the change of the microsurroundings.

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スピクリスボール酸から誘導されたローダミン型染料の合成と性質

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要 旨

糸状菌 Penicillium spiculisporum の生産するスピクリスボール酸（1）は、分子内にカルボキシル基、ラクトン環及び長鎖アルキル基を併せ持つ多官能性物質であり、収量（培養液中 110 g/l）も大きく、バイオサーフェークタントなどとして注目されている。ここでは、1にローダミン型色素構造を導入することを試み、合成法と生成した新しい色素の構造、色、蛍光性及び両親媒性などについて検討を加えた。

まず1を加熱、脱水して酸無水物（II）としたのも、次にこのIIにm-ジチルアミノフェノールを加え、加熱融解し、黒味赤色の相当する色素ベースを得た。この色素ベースを所定の塩酸水溶液に溶解し、カチオン塩すると鮮明な赤紫色の溶液が得られた。試料の λmax（希塩酸溶液）は 571 nm（吸光 589 nm）を示した。

本色素の特徴は両親媒性構造を有するため界面活性を示すことがある。水溶液の表面張力を対数曲線には屈折点があり、ミセルを形成する。臨界ミセル濃度（cmc）以上での平衡表面張力は 35.1 mN/m を示した。応用面で染料としては、ナイロン、羊毛及び綿に対し着色性（赤紫色）を示した。また、界面活性な蛍光色素であるため、バイオセンサーなどミクロな環境指示薬として期待される。

（1987 年 10 月、色材研究発表会（創立 60 周年）にて、一部発表）