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Cycloaromatization and DNA cleavage of novel non-conjugated aromatic enetetrayne systems

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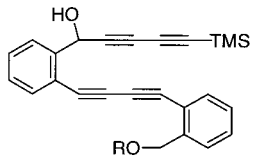
The novel, non-conjugated aromatic enetetrayne (**2**) underwent thermal cycloaromatization reaction to give polyphenylene derivative **6**, forming a methyl cation as an active intermediate, and showed DNA-cleaving activity.

1. Introduction

Ever since the disclosure of the spectacular structures and mode of action of the natural enediyne antitumor antibiotics, much attention has been focused on the preparation of simple model compounds [1–3] with analogous antitumor and antibiotic activity. We recently reported synthesis and cycloaromatization of the new enetetraynes (**1a**–**f**) [4, 5] and DNA-cleaving activity of **1f** [5]. We report herein an intriguing example of the generation of methyl cation capable of methylating DNA by cycloaromatization of **2** bearing a pyridine ring instead of the benzene ring of **1**, the solubility in water of which will be improved by introduction of a nitrogen atom.



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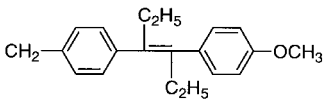
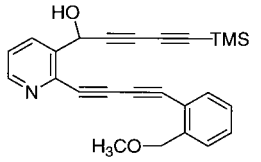
a: R=THP, **b**: R=CH_3, **c**: R=$CH_2CH{=}CH_2$,
d: R=$CH_2C_6H_5$, **e**: R=$CH(C_6H_5)_2$,
f: R=





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2. Investigations, results and discussion

2.1. Chemistry

Compound **2** was easily prepared in two steps (Scheme 1). The $PdCl_2(PPh_3)_2$-catalyzed coupling [6] of **3** [7] with **4** [5] afforded the alkynylated coupling product **5** in 85% yield. Reaction of **5** with 4-trimethylsilylbutadiyne-1,3-yl lithium [5] resulted in the formation of **2** in 87% yield. As **2** is cyclized gradually to the corresponding polycyclic compound **6** at room temperature, isolation, purification and storage of this compound should be done with care, keeping the temperature below 0 °C to avoid the following cyclization.

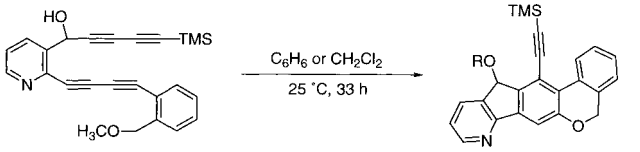
2.2. Thermal cycloaromatization of 2

Thermolysis of **2** (1.8 mM) in purified benzene at 25 °C for 33 h afforded **6** in 41% yield along with its *O*-methyl ether derivative **7** in 22% yield which may be produced by methylation of **6** with a methyl cation or carbene generated from **2** (Scheme 2). When this reaction was carried out in dichloromethane under the same conditions, **6** was obtained in 52% yield along with **7** in 6% yield. Although we have not enough evidence to explain the formation of the methyl cation, the finding suggests the following mechanism for this cyclization reaction and the formation of the methyl cation (Scheme 3). At the initial step, **2** forms an outer-biradical **A** which has been not captured yet. The biradical **A** is converted into a 1,2-benzyne intermediate **B** by intramolecular cyclization. The following cyclization reaction of **B** gives an ionic intermediate **C** which will be converted into two ionic intermediates, a carbanion **D** and a methyl cation. The carbanion **D** is led to the final product **6**, abstracting a hydrogen atom. The methyl cation generated reacts with **6** to give **7**. Both ionic intermediates may serve as an active species for DNA damage.

2.3. DNA-cleaving activity with 2

We next examined the DNA-cleaving properties of **2** (Fig. 1). Incubation of supercoiled circular Bluescript II KS^+ DNA (form I) (2,961 base pair) with drug **2** induced the transformation of form I DNA into nicked circular (form II) DNA at 5 μM drug concentration (lane 3).

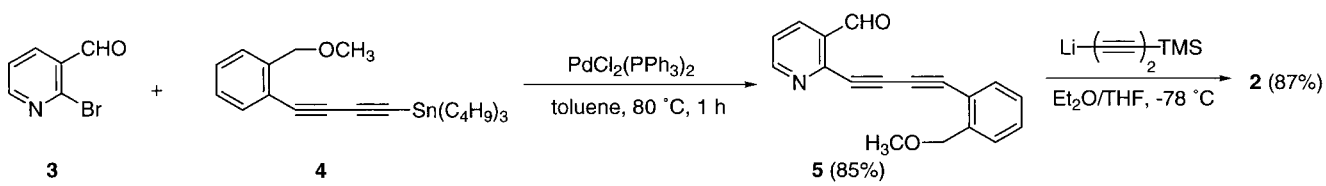
Scheme 2



2

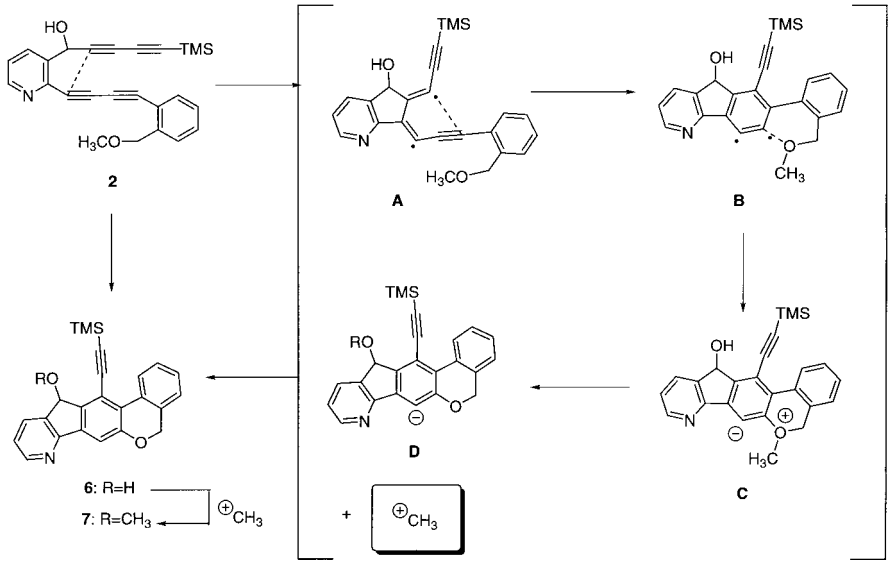
6: R=H (41%)
7: R=CH_3 (22%)

Scheme 1



3 **4** **5** (85%)

Scheme 3



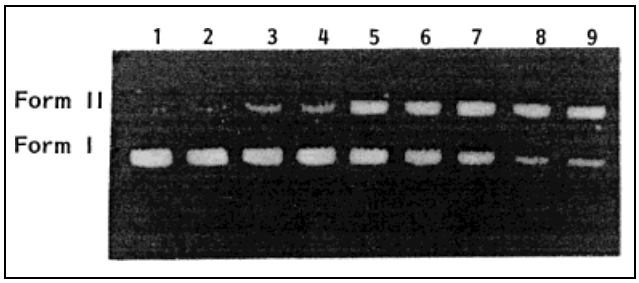


Fig. 1: Cleavage of supercoiled circular Bluescript II KS$^+$ DNA (form I) in the presence of **2**. Lane 1, DNA control; lane 2, DNA + **2** (1.0 μM); lane 3, DNA + **2** (5.0 μM); lane 4, DNA + **2** (10 μM); lane 5, DNA + **2** (50 μM); lane 6, DNA + **2** (100 μM); lane 7, DNA + **2** (500 μM); lane 8, DNA + **2** (1,000 μM); lane 9, DNA + **2** (2,000 μM)

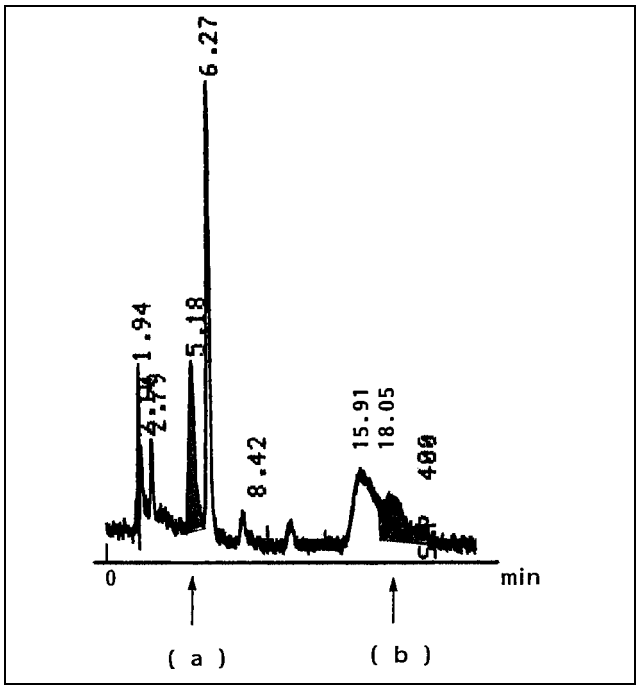
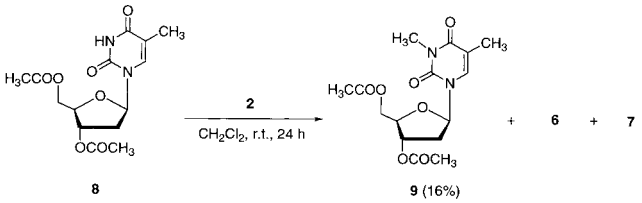


Fig. 2: HPLC-UV chromatogram of the reaction mixture obtained by reaction of **2** with calf thymus DNA. a) 1-methyladenine derivative (**13**) with retention time of 5.18 min. b) 7-methylguanine derivative (**14**) with retention time of 18.05 min

Scheme 4

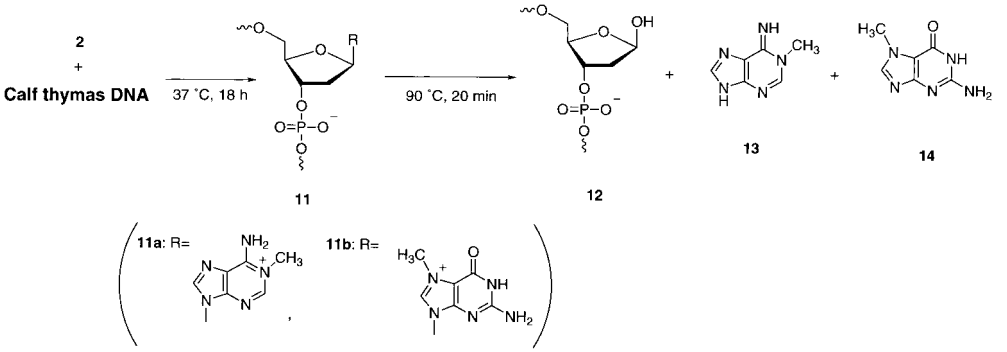


In order to study the DNA-methylating properties of the active species, the methyl cation, a solution of **2** (5 mM) and a thymidine derivative (**8**; 1 mM) in dichloromethane was stirred at 25 °C under argon in the dark, resulting in the formation of the N^3-methylated thymidine derivative **9** in 16% yield together with **6** in 50% yield and **7** in 8% yield by column chromatographic separation (Scheme 4).

Encouraged by this finding, we examined the reaction of **2** in the presence of calf thymus DNA. A mixture of **2** (1.3 mg, 3.3 mM) and calf thymus DNA (10 mg) in 10% dimethyl sulfoxide (DMSO) in a Tris-acetate buffer (pH 5.0) was incubated at 37 °C for 18 h. The modified DNA was recovered from the reaction mixture by ethanol-precipitation according to a common procedure. Compounds **6** and **7** were completely removed by this procedure. The precipitates obtained were dissolved into 1M Tris-acetate buffer (pH 5.0), and the resulting solution was heated at 90 °C for 20 min [8]. HPLC analysis of the reaction mixture revealed the presence of the 1-methyladenine derivative **13** and the 7-methylguanine derivative **14**, with retention times of 5.18 min and 18.05 min, among a few other products (Fig. 2). These results indicate that the methyl cation generated from **2** by the cycloaromatization is capable of undergoing DNA methylation at adenine N1 and at guanine N7 to produce methylated DNA (**11a** and **11b**), which upon heating at 90 °C, releases adducts (**13** and **14**) *via* hydrolytic cleavage of the glycoside bond, along with the formation of a decomposition fragment (**12**, Scheme 5).

In summary, we have demonstrated that a methyl cation is effectively generated from **2** through the thermal cycloaromatization reaction. The present work provides a new and promising entry to formation of the methyl cation which is an active species for DNA damage.

Scheme 5



3. Experimental

All m.p.'s were uncorrected and were determined on a Gallenkamp apparatus. IR Spectra were recorded on a Hitachi 260-30 spectrophotometer using the KBr Wafer technique or in neat. NMR Spectra were recorded on JEOL JNM-LA400 (400 MHz) spectrometers, operating at 400 MHz for ^{1}H NMR and at 100 MHz for ^{13}C NMR. Chemical shifts are reported in δ (ppm) relative to TMS (δ = 0) for ^{1}H NMR and relative to the central $CDCl_3$ resonance (δ = 77.0) for ^{13}C NMR. MS were measured on a JEOL JMS-600. Elemental analyses were determined using a Perkin-Elemer 240C Microanalyzer; all results were within the acceptable range (±0.3). All reactions were carried out under argon atmosphere, using dry and freshly distilled solvents under anh. conditions unless otherwise specified.

3.1. 1-(2(-(4-(2-Methoxymethyl)phenyl)buta-1,3-diynyl)(3-pyridyl)-6,6-dimethyl-6-silahepta-2,4-diyn-1-ol (2)

4-Trimethylsilylbuta-1,3-diynyl lithium, prepared from 1,4-bis(trimethylsilyl)buta-1,3-diyne (565 mg, 2.91 mmol) and 1.5 M methyllithium-lithium bromide in Et_2O (0.97 ml, 1.45 mmol) according to the literature [9], was added to a solution of **5** (200 mg, 0.73 mmol) in Et_2O (4 ml) at −20 °C. The resulting mixture was stirred for 5 min and the reaction was quenched with saturated aqueous NH_4Cl. The mixture was extracted with Et_2O and the extract was dried over $MgSO_4$. After removal of the solvent, the oily residue was purified by silica gel column chromatography with a mixed solvent of hexane and AcOEt as an eluent to give pure **2** (250 mg) as a pale yellow oil in 87% yield. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 0.19 (9 H, s), 3.47 (3 H, s), 3.66 (1 H, s), 4.64 (2 H, s), 5.98 (1 H, s), 7.26 (1 H, t, J = 7.1 Hz), 7.35 (1 H, dd, J = 4.6, 7.8 Hz), 7.40 (1 H, t, J = 7.6 Hz), 7.48 (1 H, d, J = 7.8 Hz), 7.54 (1 H, d, J = 7.8 Hz), 8.05 (1 H, dd, J = 7.8, 1.5 Hz), 8.55 (1 H, dd, J = 1.5, 7.9 Hz). ^{13}C NMR (100 MHz, $CDCl_3$) δ: −0.59, 58.68, 61.74, 71.44, 72.39, 75.63, 78.15, 78.60, 81.71, 86.88, 89.16, 119.56, 123.94, 127.41, 127.54, 129.90, 133.32, 134.76, 139.03, 140.10, 141.98, 150.02. As **2** is cyclized gradually to the corresponding polycyclic compound **6** at room temperature, isolation, purification and storage of this compound should be done with care, keeping the temperature below 0 °C to avoid the following cyclization.

3.2. 2-{4-(2-Methyoxymethyl)phenyl}buta-1,3-diynyl}pridine-3-carbaldehyde (5)

To a solution of 1-methoxymethyl-2-(4-trimethylsilylbuta-1,3-diynyl)benzene [3] (977 mg, 4.03 mmol) in CH_3OH (5 ml) was added K_2CO_3 (56 mg, 0.43 mmol) at 0 °C with care under vigorous stirring. The reaction mixture was neutralized with 1N HCl and extracted with Et_2O. The extract was dried over $MgSO_4$ and evaporated in vacuo. The residue was purified by silica gel column chromatography with C_6H_6 as an eluent. Removal of 90% volume of the solvent gave a residue containing 1-methoxymethyl-2-buta-1,3-diynylbenzene which was added to a solution of tributyltin chloride (1.46 ml, 5.38 mmol) in diisopropylamine (10 ml) and the mixture was stirred for 2 h at room temperature. After removal of the diisopropylamine, the residue was diluted with C_6H_6 and filtered on celite. The filtrate was evaporated in vacuo to give **4**. A mixture of **3** (500 mg, 2.69 mmol) [3], **4** and $PdCl_2(PPh_3)_2$ (94 mg, 0.13 mmol) in toluene (15 ml) was heated at 80 °C for 1 h. After addition of 10% aqueous KF solution, the mixture was stirred for 30 min at room temperature and filtered on celite. The filtrate was extracted with C_6H_6 and the extract was dried over $MgSO_4$. After removal of the solvent, the oily residue was purified by silica gel column chromatography with C_6H_6 as an eluent to give pure **5** (632 mg) as pale yellow powders in 85% yield; m.p. 80.0–80.9 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ; 3.49 (3 H, s), 4.66 (2 H, s), 7.29 (1 H, t, J = 7.6 Hz), 7.43 (1 H, t, J = 7.3 Hz), 7.45 (1 H, t, J = 7.8 Hz), 7.51 (1 H, d, J = 7.8 Hz), 7.58 (1 H, d, J = 7.6 Hz), 8.22 (1 H, d, J = 7.8 Hz), 8.83 (1 H, d, J = 4.6 Hz), 10.57 (1 H, s). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 58.73, 72.38, 77.65, 80.00, 82.54, 100.54, 119.16, 123.80, 127.48, 127.61, 130.22, 133.25, 133.46, 134.85, 142.20, 144.87, 154.63, 190.14. IR (KBr) cm^{-1}: 2350, 2230, 1705. FABMS m/z: 276 (MH^+), 244.
$C_{18}H_{13}NO_2$

3.3. 7-(3,3-Dimethyl-3-silabut-1-ynyl)pyridino[3′,2′-3,2]indeno[5,6-c]isochromen-8-ol (6) *and 7-(3,3-Dimethyl-3-silabut-1-ynyl)-8-methoxypyridino[3′,2′-3,2]-indeno[5,6-c]isochromene* (7)

A solution of **2** (250 mg, 0.63 mmol) in C_6H_6 (1.2 l) was stirred for 33 h at 25 °C in the dark. After removal of the solvent in vacuo, the oily residue was purified by silica gel column chromatography with a mixed solvent of hexane and AcOEt as an eluent to give pure **6** (99 mg) as yellow powders and **7** (40 mg) as yellow powders.
6: Yield 41%; m.p. 151.3–152.5 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 0.36 (9 H, s), 3.46 (1 H, br), 5.05 (2 H, s), 5.83 (1 H, s), 7.22 (2 H, m), 7.33 (1 H, t, J = 7.6 Hz), 7.39 (1 H, t, J = 7.6 Hz), 7.59 (1 H, s), 7.94 (1 H, d, J = 7.6 Hz), 8.56 (1 H, m), 8.77 (1 H, d, J = 7.6 Hz). ^{13}C NMR (100 MHz, $CDCl_3$) δ: −0.38, 69.00, 72.48, 102.46, 105.00, 110.94, 115.60, 122.47, 124.57, 125.26, 125.61, 127.71, 128.35, 129.43, 132.72, 132.78, 139.17, 140.18, 144.60, 150.18, 151.22, 158.16. IR (KBr) cm^{-1}: 2170, 2350, 3450. FAB MS m/z: 384 (MH^+), 366, 154.
$C_{24}H_{21}NOSi$
7: Yield 22%; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 0.33 (9 H, s), 3.30 (3 H, s), 5.05 (2 H, d-like, J = 4.9 Hz), 5.65 (1 H, s), 7.22 (2 H, m), 7.35 (1 H, t, J = 7.3 Hz), 7.39 (1 H, t, J = 7.6 Hz), 7.60 (1 H, s), 7.92 (1 H, d, J = 7.6 Hz), 8.59 (1 H, d, J = 4.9 Hz), 8.90 (1 H, d, J = 7.8). ^{13}C NMR (100 MHz, $CDCl_3$) δ: −0.31, 54.46, 69.07, 79.57, 101.76, 104.43, 110.34, 117.59, 122.31, 124.46, 125.69, 126.12, 127.59, 128.22, 129.60, 132.52, 132.85, 137.98, 140.77, 141.04, 150.31, 157.45, 158.85. IR ($CHCl_3$) cm^{-1}: 2370, 2340, 2170. FAB MS m/z: 398 (MH^+), 366.

3.4. 3′,5′-Diacetylthymidine (8)

Compound **8** was prepared in 79% from commercially available thymidine (1.0 g, 4.13 mmol) and acetic anhydride (50 ml) according to the literature [10].

3.5. 3′,5′-Diacetyl-3-methylthymidine (9)

Sodium hydride (60% dispersion in mineral oil; 12.3 mg, 0.31 mmol) and methyl iodide (20 ml, 0.62 mmol) was added to a solution of **8** (100 mg, 0.31 mmol) in DMF (2 ml) at 0 °C. The mixture was stirred for 30 min at the same temperature, poured into ice-water and extracted with CH_2Cl_2. The extract was washed with H_2O and dried over $MgSO_4$. After removal of the solvent, the residue was purified by silica gel column chromatography with a mixed solvent of hexane and AcOEt as an eluent to give pure **9** (64 mg) in 61% yield [11]. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 1.96 (3 H, s), 2.11 (3 H, s), 2.13 (3 H, s), 2.16 (2 H, dd, J = 7.1, 14.9 Hz), 2.49 (1 H, m), 3.35 (3 H, s), 4.25 (1 H, m), 4.35 (2 H, d, J = 3.9), 5.22 (1 H, d, J = 6.6 Hz), 6.34 (1 H, dd, J = 5.9, 8.3 Hz).

3.6. Reaction of 2 *with supercoiled circular Bluescript II KS$^+$ DNA (form I; 2,961 base pairs)*

DNA-Cleaving tests were carried out in Eppen-tubes. Drug **2** in DMSO (5 μl), the DNA (form I) (16.0 μg), 1M Tris-acetate buffer (pH 5.0) (2.5 μl) and DDW (41.5 μl) were replaced in an Eppen-tube. The mixture was stirred with a vortex mixer. After centrifuging, the resulting mixture containing the DNA (form I) and varying concentrations of **2** in 10% DMSO in 1M Tris-acetate buffer solution were incubated for 3 h at 37 °C. 10M NH_4OAc (30 μl), 99.5% EtOH (200 μl) and glycogen (2 μl) were added to the reaction mixture. The resulting mixture was stirred with a vortex mixer, allowed to stand at −20 °C for 15 min and then centrifuged at 15,000 rpm for 15 min at 5 °C to give a pasty cake which was dried in vacuo. The pasty cake dried was dissolved into DDW. After addition of

the loading solution, the mixture was stirred for 20 min and centrifuged at 15,000 rpm for 15 min at 5 °C. The solution obtained was analyzed by electrophoresis (0.7% agarose gel, ethidium bromide stain). Results are shown in Fig. 1.

3.7. Reaction of **2** *with* **8**

A mixture of **2** (150 mg, 0.38 mmol) and **8** (25 mg, 0.077 mol) in CH_2Cl_2 (75 ml) was stirred for 24 h at 25 °C. After removal of the solvent, the residue was purified by silica gel column chromatography with a mixed solvent of hexane and AcOEt as an eluent to give pure **9** in 16% yield along with **6** and **7** in 50% and 8% yields, respectively. The structure of each compound was assigned on the spectral data, compared with those of **6**, **7** and **9** prepared by alternative methods.

3.8. Reaction of **2** *with calf thymus DNA*

A mixture of **2** (1.3 mg) in DMSO (100 μl) and commercially available calf thymus DNA (10 mg) in 1M Tris-acetate buffer (pH 5.0) (50 μl) and DDW (850 ml) in an Eppen-tube was incubated for 18 h at 37 °C. The reaction mixture (200 μl) was treated with a mixture of EtOH (800 μl) and 10 M ammonium acetate (120 μl). Centrifugation of the mixture at 15000 rpm for 15 min at 5 °C gave a pasty cake which was dissolved into 1M Tris-acetate buffer (pH 5.0) (1 ml). The mixture was heated at 90 °C for 20 min and analyzed by HPLC. Results are shown in Fig. 2.

3.9. HPLC Apparatus and chromatographic conditions

HPLC was performed with a Hitachi 655A-12 liquid chromatograph (Tokyo, Japan) equipped with two pumps and a Hitachi 655A variable wavelength UV monitor. The analytical column was a stainless steel cartridge (4.6×150 mm^2) packed with Cosmosil $5C_{18}$ purchased from Hitachi Co. Ltd., maintained at 40 °C in a water-thermostat. The mobile phase was 0.05 M ammonium formate in H_2O. The flow rate was 0.7 ml/min. The detection wavelength was 258 nm. Retention times and peak areas were measured by a Hitachi D-2000 chromato-integrator.

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