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Note

1-Phenyl-3-(α- and -β-D-threofuranosyl-pyrazolo[3,4-*b*]-quinoxaline C-nucleoside analogues. Synthesis and anomeric configuration assignment by CD and ^{1}H NMR spectroscopy ☆,☆☆

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Some pyrazolo[3,4-*b*]quinoxalines have tuberculostatic activity in vitro [3], while others show antifungal activity [4]. We have been interested recently in the synthesis of *C*-nucleoside analogues having the pyrazolo[3,4-*b*]quinoxaline base moiety and their substituted analogues [2,5–7] for biological evaluation. These cyclic compounds are obtained by acid-catalyzed dehydration of their acyclic precursors, usually as anomeric mixtures, and the determination of the anomeric configuration is a problem of special importance. High-resolution NMR and CD spectra [5] have been used for this purpose. The previously studied analogues were prepared by dehydrative cyclization of acyclic precursors having the same D-*arabino*-polyhydroxyalkyl side chain, but with different substituents at the base moiety. Acid-catalyzed dehydration of these compounds gives predominantly the β-anomeric *C*-nucleoside. In this work, a stereoisomeric pyrazolo[3,4-*b*]quinoxaline analogue having the *lyxo* configuration of the side chain was prepared, and the cyclized *C*-nucleoside products were isolated and their anomeric configurations determined by ^{1}H NMR and CD spectroscopy.

1. Discussion

Dehydration of 1-phenyl-3-(D-*lyxo*-tetritol-1-yl)pyrazolo-[3,4-*b*]quinoxaline (**1**) with concentrated hydrochloric acid or trifluoroacetic acid gave a mixture of

☆ For a preliminary report see Ref 1. *C*-Nucleoside Pyrazolo[3,4-b]quinoxaline Analogues Part V. For Part IV, see Ref. 2.

☆☆ Dedicated to Professor Leroy B. Townsend on the occasion of his 60th birthday.

[1] Deceased 1992.

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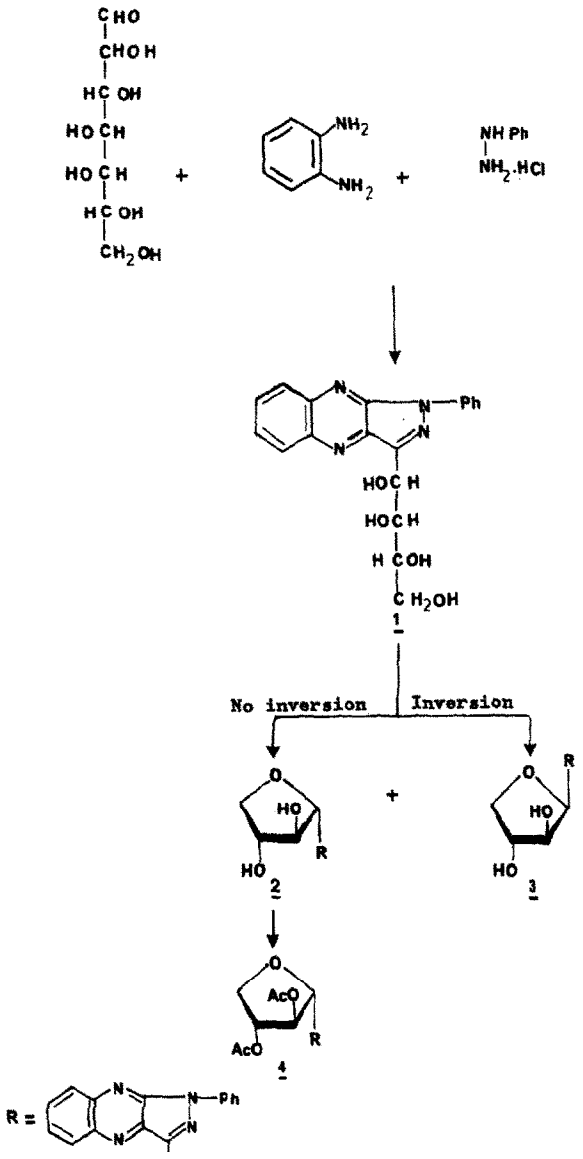

Scheme 1.

anomeric *C*-nucleoside analogues; namely, 1-phenyl-3-α-D-threofuranosylpyrazolo-[3,4-*b*]quinoxaline (**2**) and 1-phenyl-3-β-D-threofuranosylpyrazolo[3,4-*b*]quinoxaline (**3**) (Scheme 1), in the ratio of 3:1. They were separated by chromatography. Compound **2** showed the anomeric proton as a doublet at δ 5.302 having the $J_{1',2'}$ coupling constant of 5.4 Hz. This large value of the coupling constant does not define the anomeric configuration [8–12]. Likewise, the acetyl derivative **4** showed the anomeric proton H-1′ at δ 5.578 with a coupling constant of $J_{1',2'}$ 4.5 Hz.

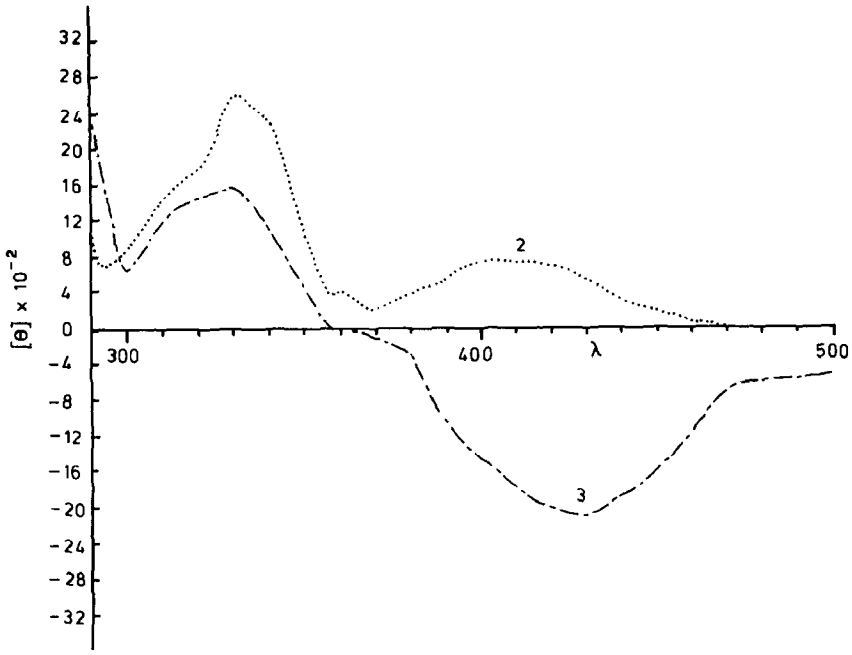

Fig. 1. CD spectra of 1-phenyl-3-α-D-threofuranosylpyrazolo[3,4-*b*]quinoxaline (**2**) (···) and 1-phenyl-3-β-D-threofuranosylpyrazolo[3,4-*b*]quinoxaline (**3**) (–·–).

Compound **3** showed the anomeric proton as a doublet at δ 5.810 having a $J_{1',2'}$ coupling constant of 3.9 Hz. The assignment of the anomeric configuration of **2** and **3** from these spin–spin coupling-constant values between H-1′ and H-2′ was more difficult since these values are not consistently diagnostic. Similar examples are reported where the assignment of the anomeric configuration to *C*-glycofuranosyl compounds on the basis of ^{1}H NMR coupling constants is unreliable [13–15]. However, having the two anomers on hand, the anomeric assignment can be determined from the chemical-shift values for their anomeric protons (H-1′). It is a general rule [10] that the anomeric proton signal of the *cis* H-1′, H-2′ isomer usually appears at lower field than that of the *trans* isomer. Accordingly, **3** having the anomeric proton at lower field (δ 5.810) was assigned the β-D-*threo* configuration, and **2** having the lower value (δ 5.302) was given the α-D-*threo* configuration. The upfield shift of the anomeric proton of **2** is attributed to the shielding of H-1′ by the *cis*-hydroxyl group at C-2′ which is also commonly seen in ribofuranosyl *N*- and *C*-nucleoside compounds [10].

Additional evidence supporting the ^{1}H NMR assignment for the anomeric configuration of **2** and **3** was obtained from their CD spectra (see Fig. 1). Compounds **2** and **3** showed multiple Cotton effects with different spectral patterns from that of the acyclic analogue [16] **1** due to the fact that the cyclic structure of **2** and **3** is not frozen in one conformation as is the planar zigzag structure of the acyclic derivative **1**. The Cotton effect at the long wavelength

absorption is due to an $n-\pi^*$ transition and is manifested by the configuration of the carbon atom which is α to the heterocyclic ring. A positive Cotton effect at the long wavelength absorption indicates the L-*glycero* configuration of C-1′, and vice versa [16]. However, **2** showed a positive Cotton effect at 360–500 nm, similar in sign to that of the precursor pyrazolo[3,4-*b*]quinoxaline [16] **1**, suggesting the same D-*lyxo* configuration in the Fischer projection formula for **1** and **2**. Compound **2** was obtained from **1** without inversion in the configuration of C-1′, that is, having the α-D-*threo* configuration of the furanosyl group formed. On the other hand, **3** showed a negative Cotton effect of opposite sign to that of the precursor pyrazolo[3,4-*b*]quinoxaline **1**, suggesting the D-*xylo* configuration for the Fischer projection formula of **3**, which was obtained from **1** with inversion of the configuration of C-1′, i.e., having the β-D-*threo* configuration. This was consistent with the optical properties at the D line for compounds **2** and **3**. In accordance with the Hudson isorotation rule [17], **2** showed the larger positive specific rotation ($[\alpha]_D^{20}$ in MeOH: **2**, +79.0°; **3**, +39.4°).

Acid-catalyzed dehydration of tetrahydroxybutylpyrazolo[3,4-*b*]quinoxalines and osazones [18] is a stereoselective process with the production of the preponderant isomer as the stereofavored isomer having a *trans* relationship between the base moiety and the 2′-OH group. Compound **2** was obtained as the preponderant isomer in accord with these stereo requirements. In addition, **3** having a *cis* relationship between the base moiety and 2′-OH group was obtained in relatively higher yield than the corresponding isomers obtained from the dehydration of the acyclic analogues having the D-*arabino* configuration [5–7]. This is due to the effect of configuration of the acyclic side-chain on the course of the dehydrative cyclization process. A similar correlation was found for acid-catalyzed dehydrative cyclization of simple pentitols [19].

The dehydrative cyclization of **1** in basic medium with *p*-toluenesulfonyl chloride (1 : 1.4 mol equiv) in pyridine is more stereospecific, with the formation of the preponderant isomer **2** in higher proportion (the ratio of **2**:**3** is 5 : 1 as determined by TLC). However, the overall yield of **2** by this method is lower (26%) due to the formation of partialy tosylated derivatives, and the recovery of some starting material (see Experimental section).

The formation of **2** as the preponderant isomer from **1**, in basic medium without inversion of the configuration of C-1′, may be explained through the initial formation of the kinetically formed 4′-monotosyl derivative which undergoes S_N2 attack from the back by the favorably disposed 2′-hydroxyl group, giving the thermodynamically stable cyclic analogue **2**.

The mass spectrum of **1** showed the molecular-ion peak at m/z 366, and the base peak at m/z 275 corresponds to the fragment BCHOH. Compounds **2** and **3** showed the molecular-ion peak at m/z 348. The base peak for **2** at m/z 77 corresponds to the Ph group. The fragment BCHOH characteristic for *C*-nucleoside analogues [6] was observed at m/z 275 as an intense peak for **2** and as the base peak for **3**.

The mass spectrum of compound **4** showed the protonated molecular ion (M + 1) at m/z 433. However, its EI mass spectrum showed the highest mass peak

at m/z 372, corresponding to the fragment M − AcOH. The base peak at m/z 43 corresponds to the CH_2CO group, as expected for the mass spectra of acetylated carbohydrates.

2. Experimental

General.—Melting points are uncorrected. Evaporations were performed under diminished pressure at < 60°C. Thin-layer chromatography (TLC) was on silica gel (Kiesel gel G, Merck) with solvent *A*, 10:1 $CHCl_3$–MeOH, and solvent *B*, 3:1 benzene–EtOH. The yellow spots were detected with UV light (bright fluorescence). IR absorption spectra were recorded with a Unicam SP 1025 instrument. UV absorption spectra were recorded for solutions in 1,4-dioxane with a Perkin–Elmer Lambda 4B instrument. Circular dichroism measurements were recorded with a Cary 60 spectropolarimeter, for solutions in 1,4-dioxane, at a dynode voltage not more than 0.75 kV. ^{1}H NMR spectra were recorded with Bruker WM (400 MHz) and Varian EM-390 (90 MHz) instruments using Me_4Si as the internal reference standard. Mass spectra were obtained at 70 eV with Dupont MS 21-492B and AEI MS 902 instruments. Combustion analyses were performed in the Department of Chemistry, Purdue University and the Chemistry Department, Faculty of Science, Cairo University, Cairo, Egypt.

*1-Phenyl-3-(*D-lyxo-*tetritol-1-yl)pyrazolo[3,4-*b*]quinoxaline* (**1**).—A solution of a syrupy mixture of D-*glycero*-L-*manno*- and D-*glycero*-L-*gluco*-heptoses [20] (15 g) in water (200 mL), was heated with *o*-phenylenediamine (6.6 g), phenylhydrazine hydrochloride (42 g), and AcOH (15.6 mL) in a sealed flask for 8 h in a boiling-water bath. The flask was cooled and opened, and the yellow precipitate was filtered off, washed successively with water, 50% MeOH, ether, and dried; yield 7 g. Recrystallization from PrOH gave yellow needles; mp 215–216°C; $[\alpha]_D^{20}$ +15.9° (*c* 2.13, MeOH); $\lambda_{max}^{1,4\text{-dioxane}}$ 268, 333, and 424 nm (log ϵ 4.6, 4.0, and 3.6; ν_{max}^{KBr} 3120 (OH) and 1600 cm^{-1} (C=N); ^{1}H NMR data (see ref 21); mass spectral data: m/z 367 (0.8, M + 1), 366 (3, M), 305 (3), 289 (3), 277 (15), 276 (83, BCH_2OH, where B = 1-phenylpyrazolo[3,4-*b*]quinoxaline moiety), 275 (100, BCHOH), 274 (8, BCHO), 254 (4), 248 (7), 247 (35, BH_2), 246 (6, BH), 245 (15, B), 220 (17, BH_2 − HCN), 92 (4, PhNH), 91 (3, PhN), and 77 (14, Ph); accurate measurement of the molecular-ion peak: Calcd for $C_{19}H_{18}N_4O_4$: 366.1328: Found 366.1327; circular dichroism data in 1,4-dioxane (*c*, 0.682 mg/mL) at 22°C; 472 ([Θ] 0), 448 (+107), 438 (0), 430 (−430), 420 (−698), 405 (−913), 390 (−806), 380 (−644), 370 [−591(sh)], 350 (−698), 340 (−537), 315 (−322), 295 (−806), 283 (0), 280 (+537). Anal. Calcd for $C_{19}H_{18}N_4O_4$: C, 62.27; H, 4.95; N, 15.30. Found: C, 62.25; H, 5.07; N, 15.32.

*Conversion of 1 into 1-phenyl-α- and -β-*D-*threofuranosylpyrazolo[3,4-*b*]quinoxalines.*—A suspension of **1** (0.5 g) in concd HCl (5 mL) was heated in a sealed flask for 1 h in a boiling-water bath. The flask was cooled, opened, and the solution was evaporated till dry. Traces of acid were removed by coevaporation with MeOH (3 × 50 mL) and then with toluene (3 × 20 mL). The yellow precipitate formed was

taken up in water, filtered, washed with water, and dried; yield 0.31 g (65%). TLC (solvent *A*) showed two spots in the ratio of 3:1 (as seen from the relative intensity of spots under UV); R_f 0.59 and 0.48, respectively.

Heating of **1** (0.5 g) with CF_3CO_2H (5 mL) under reflux on a boiling-water bath for 4 h, evaporation of the acid under vacuum, and removal of traces of the acid by coevaporation with toluene, gave a yellow precipitate that showed the same composition in TLC. The two spots were separated by preparative TLC using solvent *A* as eluent.

*1-Phenyl-3-α-*D*-threofuranosylpyrazolo[3,4-*b*]quinoxaline* (**2**).—This compound was separated as the faster moving preponderant spot and recrystallized from MeOH as yellow needles; mp 246–248°C; R_f 0.59 (solvent *A*) and 0.58 (solvent *B*); $[\alpha]_D^{20}$ +79.0° (*c* 0.50, pyridine); υ_{max}^{KBr} 3460 (OH), 1620 (C=N), and 1510, 770 cm^{-1} (Ph); ^1H NMR data: (400 MHz, Me_2SO-d_6): δ 4.092 (dd, 1 H, $J_{3',4''}$, 4.3, $J_{4',4''}$ 9.0 Hz, H-4″), 4.261 (dd, 1 H, $J_{3',4'}$ 5.6 Hz, H-4′), 4.371 (m, 1 H, H-3′), 4.975 (dd, 1 H, *J* 4.8 Hz, H-2′), 5.302 (d, 1 H, $J_{1',2'}$ 5.4 Hz, H-1′), 5.554 (d, 1 H, *J* 5.2 Hz, HO-2′), 5.752 (d, 1 H, *J* 5.1 Hz, HO-2′), 7.250 (t, 1 H, H-*p*), 7.780 (t, 2 H, H-*m*), 8.042 (t, 1 H, H-*o*), 8.135 (t, 1 H, *J* 7.1, 7.4 Hz, H-*o*′), 8.132 (d, 1 H, *J* 8.6 Hz, H-7), 8.430 (d, 1 H, H-6), 8.501 (s, 1 H, H-8), 8.521 (s, 1 H, H-5). After addition of CD_3CO_2D, the two OH proton signals disappeared; mass spectral data: *m/z* 349 (6, MH), 348 (12, M), 301 (3), 289 (13, $BHCH_2CHO$), 287 (4, BCH_2CO), 276 (5, BHCHOH), 275 (28*, BCHOH), 259 (5, BCH_2), 247 (5, BH_2), 245 (12, B), 220 (15, BH_2 – HCN), 129 (5), 102 (12), 91 (7, PhN), 77 (100, Ph), 76 (11), 65 (8), 64 (9), 55 (13), and 51 (42); circular dichroism data in 1,4-dioxane (*c*, 0.04 mg/mL) at 22°C; 460 ([Θ] 0), 420 (+626), 410 (+696), 380 (+348), 370 (+174), 360 (+348), 340 [+2262(sh)], 330 (+2610), 320 [+1740(sh)], and 300 (+870). Anal. Calcd for $C_{19}H_{16}N_4O_3$: C, 65.49; H, 4.63; N, 16.09. Found: C, 65.60; H, 4.60; N, 15.59.

*1-Phenyl-3-β-*D*-threofuranosylpyrazolo[3,4-*b*]quinoxaline* (**3**).—This compound was isolated by PLC as the slower moving spot and recrystallized from dil MeOH as pale yellow needles; mp 280–281°C; $[\alpha]_D^{20}$ +39.4° (*c* 0.63, pyridine); R_f 0.48 (solvent *A*) and 0.53 (solvent *B*); υ_{max}^{KBr} 3420 (OH), 1600 (C=N), 1500 and 750 cm^{-1} (Ph); ^1H NMR data (400 MHz, Me_2SO-d_6): 3.905 (dd, 1 H, H-4″, $J_{3',4''}$ 1.1, $J_{4',4''}$ 8.9 Hz, H-4″), 4.401 (m, 1 H, H-3′), 4.471 (dd, 1 H, $J_{3',4'}$ 4.0 Hz, H-4′), 5.083 (d, 1 H, $J_{3',OH}$ 4.3 Hz, HO-3′), 5.526 (d, 1 H, $J_{2',OH}$ 3.5 Hz, HO-2′), 5.810 (d, 1 H, $J_{1',2'}$ 3.9 Hz, H-1′), 7.462–4.492 (m, 1 H, H-*p*), 7.725–7.765 (m, 1 H, H-*m*), 7.973–8.014 (m 1 H, H-*o*), 8.074–8.116 (m, 1 H, H-*o*′), 8.351 (dd, 1 H, *J* 1.0, 8.5 Hz, H-7), 8.414 (dd, 1 H, H-6), 8.500 (d, 1 H, *J* 8.7 Hz, H-8), and 8.521 (d, 1 H, *J* 1.0 Hz, H-5). After addition of CD_3CO_2D, the two OH proton signals disappeared; mass spectral data: *m/z* 349 (4, MH), 348 (27, M), 301 (3), 289 (18, $BHCH_2CHO$), 276 (14, BHCHOH), 275 (100, BCHOH), 259 (5, BCH_2CHOH), 247 (7, BH_2), 246 (3, BH), 245 (14, B), 220 (18, BH_2 – HCN), 188 (27), 91 (7, PhN), 77 (49), and 51 (18); circular dichroism data in 1,4-dioxane (*c*, 0.036 mg/mL) at 22°C; 500 ([Θ] – 348), 470 (–725), 440 (–1933), 430 (–2127), 400 (–1547), 380 (–773), 355 (0), 350 (+387), 330 (+1550), 310 [+1160(sh)], and 300 (+580). Anal. Calcd for $C_{19}H_{16}N_4O_3$: C, 65.49; H, 4.63; N, 16.10. Found: C, 65.10; H, 4.58; N, 15.76.

*3-(2,3-Di-*O*-acetyl-α-*D*-threofuranosyl)-1-phenylpyrazolo[3,4-*b*]quinoxaline* (**4**).—

A solution of **2** (0.1 g) in pyridine (3 mL) was treated with Ac_2O (3 mL) for 24 h at room temperature. It was then poured into crushed ice, and the acetate was filtered off, washed with water, and dried; yield 0.12 g. It was recrystallized from dil MeOH to give yellow needles; mp 125°C; ^1H NMR data (90 MHz, $CDCl_3$): δ 2.070 and 2.110 (d, 6 H, 2 CH_3CO), 4.300–4.400 (m, 2 H, H-4′, 4″), 5.411 (m, 1 H, H-3′), 5.578 (d, 1 H, $J_{1',2'}$ 4.5 Hz, H-1′), 6.311 (dd, 1 H, $J_{2',3'}$ 3.0 Hz, H-2′), 7.33 (m, 1 H, H-*p*), 7.533 (m, 1 H, H-*m*), 7.777 (m, 2 H, H-*o*), 8.11 (d, 1 H, H-7), 8.233 (d, 1 H, *J* 7.0 Hz, H-6), 8.367 (s, 1 H, H-8), and 8.467 (s, 1 H, H-5); mass spectral data (CI): *m/z* 433 (100, M + 1); (EI); 372 (1, M − AcOH), 314 (3, M − 2 OAc), 313 (13, M − H − 2OAc), 301 (1), 275 (1, BCHOH), 246 (1, BH), 245 (2, B), 220 (1, BH_2 − HCN), 115 (4), 77 (4, Ph), and 43 (100, CH_3CO). Anal. Calcd for $C_{23}H_{20}N_4O_5$: C, 63.87; H, 4.66; N, 12.96. Found: C, 63.50; H, 4.60; N, 12.90.

Treatment of **1** *with* p-*toluenesulfonyl chloride.*—Compound **1** (0.2 g 0.55 mmol) was dried for 4 h at 110°C/5 mtorr, and dissolved in anhyd pyridine (5 mL). The solution was treated portionwise at room temperature with *p*-toluenesulfonyl chloride (0.146 g, 1.4 mol equiv). The mixture was kept at room temperature for 24 h, a few drops of water were added, the mixture was evaporated to a syrup and traces of pyridine was removed by coevaporation with toluene. TLC indicated the presence of a small amount of starting material with the formation of two more mobile spots with R_f values identical to those of **2** and **3** in the ratio of 5:1, respectively. Unreacted **1** was removed by extraction with $CHCl_3$ (**1** was not soluble in $CHCl_3$). The $CHCl_3$ extract was filtered and evaporated to dryness to give a pale-yellow syrup that was purified by acetylation with 1:1 pyridine–Ac_2O (5 mL) for 12 h at room temperature. The mixture was poured into crushed ice and the precipitate obtained was filtered off, washed with water and dried; yield 0.2 g. The acetate mixture was deacetylated by dissolving it in MeOH (5 mL), treating it with 1 M NaOH (5 mL), keeping it overnight at room temperature, neutralizing it by bubbling in a stream of CO_2, and concentrating to a small volume. It gave a yellow precipitate that was filtered off, washed with water, and dried; yield 50 mg (26%). It was recrystallized from dilute MeOH to give yellow needless mp and mixed mp (with **2**) 246–248°C; the same R_f values (solvents *A* and *B*); identical ^1H NMR spectra; and the correct combustion analysis.

The minor isomer **3** was detected as a faint spot in the mother liquor after separation of **2** by TLC using solvent mixtures *A* and *B*.

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