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X-ray structure, NMR and stability-in-solution study of 6-(furfurylamino)-9-(tetrahydropyran-2-yl)purine – A new active compound for cosmetology

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ABSTRACT

The crystal and molecular structure of 6-(furfurylamino)-9-(tetrahydropyran-2-yl)purine (**1**) was determined at 150(2) K. The compound crystallizes in monoclinic $P2_1/c$ space group with $a = 10.5642(2)$, $b = 13.6174(3)$, $c = 10.3742(2)$ Å, $V = 1460.78(5)$ Å^3, $Z = 4$, R(F) = for 3344 unique reflections. The purine moiety and furfuryl ring are planar and the tetrahydropyran-2-yl is disordered in the ratio 1:3, probably due to the chiral carbon atom C(17). The individual ^{1}H and ^{13}C NMR signals were assigned by 2D correlation experiments such as ^{1}H–^{1}H COSY and ge-2D HSQC. Stability-in-solution was determined in methanol/water in acidic pH (3–7).

1. Introduction

Purine based derivatives called cytokinins have received considerable attention since the discovery of N^6-furfuryladenine (kinetin), a very important representative of this group [1,2]. Cytokinins are plant growth regulators that promote cell division, prevent leaf senescence and participate in almost all stages of plant development [3]. Moreover, kinetin delays the onset of aging characteristics in human fibroblasts [4] and therefore is currently included as an active agent in many cosmetic compositions [5]. Up to now, a number of cytokinin derivatives have been prepared, their biological properties have been studied and some interesting structure and activity relationship data have been obtained [6–9]. Considering particularly N(9) substitution of kinetin, various alkyls [10–13], aryls [14,15], or miscellaneously substituted ribosides [16–19] have been used as substituents. 6-(Furfurylamino)-9-(tetrahydropyran-2-yl)purine was prepared by Robins et al. (1960) by the reaction of 6-chloro-9-tetrahydropyran-2-ylpurine with N^6-furfurylamine [20]. It was later demonstrated that tetrahydropyranylation very often improves the anti-senescent properties compared to those of the free bases [6,7,21]. 6-(Furfurylamino)-9-(tetrahydropyran-2-yl)purine (**1**) was tested for its anti-senescent activity on detached soya leaves [6]. Later, it underwent a series of cytokinin assays and its biological effects were described in detail [21]. Due to structural similarity between kinetin and **1**, the compound was also tested on human fibroblasts within the frame of the anti-aging assay and it was shown to be much more effective than kinetin [21]. A clinical study of topical use of **1**, including *in vitro* studies of attachment frequency, cellular growth curves, mitochondria activity, lysosomes, accumulation of intracellular debris and overall rejuvenation, has been published recently [22]. It was established that treatment of the skin with a 0.1% concentration of this compound improved roughness and skin moisturization as well as mottled hyperpigmentation and fine wrinkles, and therefore it can serve as an anti-aging treatment [22]. The substance is currently used in several compositions for treating skin, for example in *Pyratine 6* anti-aging cream and lotion [23]. This paper reports the results of long-term research on this particular cytokinin derivative, 6-(furfurylamino)-9-(tetrahydropyran-2-yl)purine (**1**), during which not only the biological properties of the compound were studied, but also its stability-in-solution and its solid state structure. The solid state structure of the compound as well as its stability-in-solution, has not previously been described.

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2. Experimental

2.1. General

3,4-Dihydropyrane and kinetin were purchased from Sigma Aldrich and were used without further purification. Formic acid, ethyl acetate, ammonia, magnesium sulphate and methanol were purchased from Lachema and were used without further purification. Evaporation of solvents was carried out under vacuum using a rotary evaporator. Elemental analyses were determined on an EA 1112 Flash analyzer (Thermo-Finnigan). The melting point was determined on a Büchi Melting Point B-540 apparatus and was uncorrected. Thin layer chromatography (TLC) was carried out using silica gel 60 WT_{254} plates (Merck Co.). $CHCl_3$:MeOH (9:1, v:v) was used as mobile phase. The CI + mass spectra were recorded using a Polaris Q (Finnigan) mass spectrometer equipped with a Direct Insertion Probe (DIP). Compound **1** was heated in an ion source with a temperature gradient from 40 °C to 450 °C over 5 min, the mass monitoring interval was 50–1000 amu, and spectra were collected using 1.0 cyclical scans, applying 70 eV electron energy. Isobutane was used as a reagent gas at a flow-rate of 2 l/h. The mass spectrometer was directly coupled to an Xcalibur data system. ^{1}H, ^{1}H–^{1}H COSY, ^{13}C and ^{1}H–^{13}C heteronuclear single quantum coherence edited GP (ge-2D HSQC) experiments were recorded on a Bruker Avance 300 FT NMR spectrometer operating at a temperature of 300 K and at a frequency of 300.13 Hz. Samples were prepared by dissolving the substances in DMSO-d_6. Tetramethylsilane (TMS) was used as the internal reference standard. The individual ^{1}H and ^{13}C signals were assigned by 2D correlation experiments including ^{1}H–^{1}H COSY and ge-2D HSQC.

2.2. 6-(Furfurylamino)-9-(tetrahydropyran-2-yl)purine (*1*) synthesis

The compound was prepared by a previously published procedure [20] with slight modifications. 3,4-Dihydropyrane (5.4 ml, 0.059 mol), and formic acid (5 ml) were added to a stirred suspension of kinetin (5 g, 0.023 mol) in ethyl acetate (40 ml), heated to reflux (78 °C) and stirred for 3 h. The reaction mixture was cooled to 20 °C, and 25% aqueous ammonia solution (10 ml) was added to neutralize the formic acid. The organic phase was washed with water (2 × 20 ml), dried with anhydrous magnesium sulphate (5 g), and evaporated to a yellow residue (9.5 g) that was crystallized from methanol (50 ml). The resulting white crystalline product was washed with cold methanol (5 °C, 2 × 10 ml), and dried under vacuum. Yield: 5.0–5.5 g (73–80%), m.p. 135.8–137.8 °C, elemental analysis, calculated for $C_{15}H_{17}N_5O_2$ (299.34), %C 60.19, %H 5.72, %N 23.40. Found: %C 60.20, %H 5.65, %N 23.42, CI + mass spectra: $[M]^+$ = 300.35, ^{1}H NMR (300 MHz, DMSO-d_6): δ = 1.48–1.60 (m, 2H, C21H_a, C21H_b), 1.63–1.77 (m, 1H, C20H_a), 1.88–1.95 (m, 2H, C20H_b, C22H_a), 2.30 (dq, 1H, J = 11 Hz, J' = 1.9 Hz, C22H_b), 3.61–3.70 (m, 1H, C19H_a), 3.95–4.02 (m, 1H, C19H_b), 4.71 (s, 2H, C11H), 5.63 (dd, 1H, J = 11 Hz, J' = 1.9 Hz, C17H), 6.23 (d, 1H, J = 3.0 Hz, C16H), 6.35 (t, 1H, J = 3.0 Hz, C15H), 7.53 (d, 1H, J = 3.0 Hz, C14H), 8.24 (bs, 1H, N10H), 8.27 (s, 1H, C8H), 8.37 (s, 1H, C2H).

^{13}C NMR (75 MHz, DMSO-d_6): δ = 22.4 (C20), 24.4 (C21), 29.9 (C22), 36.4 (C11), 67.6 (C19), 80.8 (C17), 106.5 (C16), 110.3 (C15), 118.91 (C5), 138.8 (C2), 141.7 (C14), 152.4 (C8), 152.9 (C4), 154.12 (C6).

2.3. Crystallography

Colorless crystals suitable for X-ray diffraction study were grown by slow evaporation of an isopropanol solution at laboratory temperature. X-ray data collection was carried out at 150(2) K on a Nonius Kappa CCD diffractometer with graphite-monochromatised Mo Kα radiation, λ = 0.71073 Å. The structure was solved by direct methods (SIR97) [24] and refined by a full-matrix least-squares routine based on F^2 (SHELXL97) [25]. Non-hydrogen atoms were refined with anisotropic displacement parameters. Except for the one at N(10), the hydrogen atoms were placed in their calculated positions and refined using a riding model with U_{iso}(H) = 1.2 U_{eq} of their bonding atom. The hydrogen atom on N(10) was identified on a difference Fourier map and was refined isotropically. The refinement converged (Δ/σ_{max} = 0.001, 232 parameters) to R = 4.95% for the observed, and R = 5.53%, wR = 12.23%, GOF = 1.100 for all diffractions. The final difference map displayed no peaks of chemical significance ($\Delta\rho_{max}$ = 0.40, $\Delta\rho_{min}$ = −0.27 e Å^{-3}).

2.4. Stability of *1* in acidic solution

The pH stability of compound **1** in methanol was analyzed by HPLC–PDA (System Gold, Beckman Instruments, Fullerton, USA) and the analytes were monitored at 270 nm. 10^{-2} M solutions of compound **1** in methanol were prepared and diluted to 10^{-4} M using McIlvaine buffer solution for the appropriate pH (3–7) [26]. One hour after incubation at 25 °C, 5 µl of each prepared solution was directly injected onto a reversed phase column (Symmetry C18, 5 µm, 150 × 2.1 mm, Waters, Milford, USA). The following binary gradient was used at a flow-rate of 0.3 ml/min: 0 min, 10% A, 0–25 min, a linear gradient to 90% A, followed by 5 min isocratic elution of 90% A, where A was 100% methanol and B was 15 mM formic acid adjusted to pH 4 with ammonium. HPLC measurement of the solutions was repeated after a 24 h incubation at 25 °C. The analyses were repeated at least three times.

3. Results and discussion

The molecular structure of 6-(furfurylamino)-9-(tetrahydropyran-2-yl)purine (**1**), including atom labeling, is given in Fig. 1. Crystallographic data for **1** are given in Table 1 while the selected bond lengths, angles and torsion angles are shown in Table 2. No solvent molecules co-crystallized. We compared the structure of **1** to the structure of kinetin [27] and kinetin riboside [28] to determine the influence of the tetrahydropyran-2-yl substitution at the N(9) atom of purine moiety. The bond lengths and angles are only slightly influenced with the exception of the N(9)—C(8) and C(8)—N(7) bonds that adjoin N(9) atom. N(7)—C(8) is 1.3245(8) Å for kinetin and 1.290(1) Å and 1.312(2) for kinetin riboside and for **1**, respectively. The N(9)—C(8) bond is 1.3465(7) Å for kinetin and 1.383(2) Å and 1.360(2) Å for kinetin riboside and for **1**, respectively. The N(9) substituent also influences the surrounding angles. While the angle C(8)—N(7)—C(5) is 102.58° for kinetin, the same angle is 103.14° and 104.0(1)° for kinetin riboside and **1**, respectively. N(9)—C(4)—C(5) is 104.05° for kinetin, 106.41° for kinetin riboside and 105.6(1)° for **1**. Whilst the bonds and angles in the purine moiety are influenced only slightly, big differences were obvious in the torsion angles. The torsion angle C(6)—N(1)—C(2)—N(3) for kinetin is 0.84°, the same angle for kinetin riboside is 3.05° and 2.3(3)° for **1**. C(11)—N(10)—C(6)—C(5) torsion angle was found 174.27° for kinetin and 168.05° and 178.6(1)° for kinetin riboside and for **1**. The purine moiety is almost planar with maximum deviation from the plane of 0.0161 Å. The dihedral angle between the pyrimidine and imidazol rings in the purine moiety is 1°. The furanyl ring is almost planar with maximal deviation from the plane 0.0027 Å. Dihedral angle between furanyl ring and purine moiety is 69° and dihedral angle between furanyl and pyrimidine ring is 68.7°. The tetrahydropyran-2-yl substituent contains a chiral carbon atom whereas the substance is a racemic