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Solid-state characterization and solubility of a genistein–caffeine cocrystal

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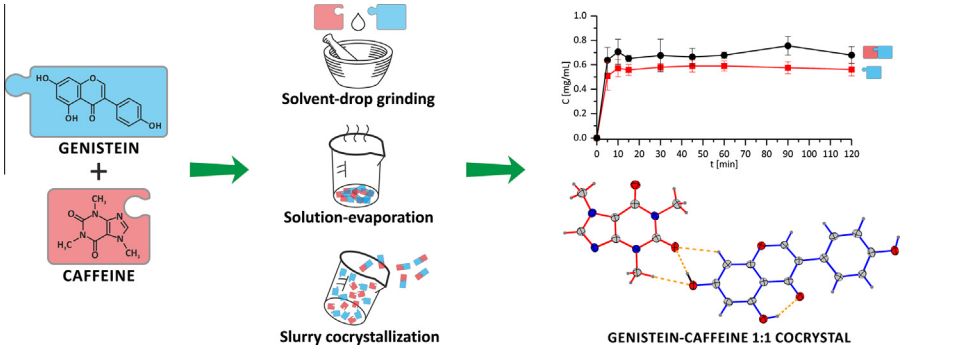
HIGHLIGHTS

- A 1:1 genistein–caffeine cocrystal was identified *via* solvent-drop grinding.
- The cocrystalline phase was grown from solution and obtained from slurry.
- Molecules form a two-dimensional, O–H···N and O–H···O hydrogen-bonded network.
- Cocrystalline phase is stable up to 247 °C.
- The genistein–caffeine 1:1 cocrystal shows improved solubility as related to parent flavonoid.

GRAPHICAL ABSTRACT



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ABSTRACT

Combination of genistein and caffeine leads to a 1:1 cocrystalline phase, which was identified by means of a solvent-drop grinding experiment and isolated afterwards in a solution-evaporation approach. Obtained cocrystal was characterized by X-ray single-crystal and powder diffraction as well as investigated in terms of thermal stability and Hirshfeld surfaces. A scale-up procedure was provided by slurry technique, enabling solubility determination. Neutral forms of both compounds cocrystallize in a common $P2_1/c$ space group of the monoclinic crystal system. Analysis of packing and interactions in the crystal lattice reveals formation of molecular layers, formed by O–H···O, O–H···N and C–H···O-type contacts between genistein and caffeine molecules, whereas stabilization of the three-dimensional crystal lattice is provided by π···π interactions. Dissolution studies in a 50:50 v/v ethanol–water medium revealed that the maximum solubility of the cocrystalline phase reached 0.861 mg/mL after 8 h, revealing some degree of enhancement as compared to parent genistein, maximum solubility of which was also reached after 8 h and equalled 0.588 mg/mL.

Introduction

A balanced diet, in which consumption of plant-derived food plays an important role, is believed to be correlated with health-promoting effects [1]. Moreover, it is a recognized observation, that some populations tend to exhibit a lowered risk of developing specific illnesses, dependent on nutritional habits [2,3]. And so, elevated consumption of soy-derived products in Asian countries is thought to be interrelated with lower rates of prostate and breast cancer morbidity as compared to western countries [3,4]. Soybeans typically contain 1–3 mg/g of isoflavonoids [5], which are low-molecular-weight polyphenolic compounds, known to exhibit various biological activities.

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One of the most abundant and widely studied isoflavonoids is genistein (4,5,7-trihydroxyisoflavone, Scheme 1). Its health-prolonging action, as related to hormonally dependent cancers, can be accounted for similitude of a potent mammalian estrogen, 17β-estradiol [6,7]. Moreover, genistein was found to inhibit tyrosine kinase [8], shows antibacterial properties [9] and is considered a promising agent in treatment of diabetes and obesity [10,11].

Similarly to most flavonoids, genistein is considered a class II agent in the biopharmaceutical classification system [12], due to low aqueous solubility ($3 \cdot 10^{-6}$ mol/dm^3) [13] and high permeability [14]. As a result of the above mentioned, application of genistein is hindered by its low bioavailability [15–17] and a search for novel forms of the compound comes into focus. Some measures to increase solubility and bioavailability of genistein were already undertaken, *i.a.* by salt formation [18], inclusion in cyclodextrin cavity [19–23], complexation with high-amylose corn starch [24], eutectic crystal melting in poly(oxyethylene) [12], matrix retention in κ-carrageenan hydrogel [25] as well as nanostructures formation [26–28].

Amongst currently applied methods of solubilisation, cocrystallization of active pharmaceutical ingredient (API) with a pharmaceutically acceptable compound [29] becomes a recognized alternative, leading to novel forms which exhibit increased solubility [30], dissolution rates [31] and bioavailability [32]. So far, cocrystallization was successfully applied to enhance bioavailability of a flavonoid quercetin [33]. As a part of our on-going research [34], a 1:1 genistein–caffeine (**GenCaf**) cocrystal was identified by solvent-drop grinding approach, obtained by solution-evaporation technique, and analysed in terms of X-ray single-crystal diffraction, Hirshfeld surfaces, thermal behaviour and solubility.

Experimental

Materials

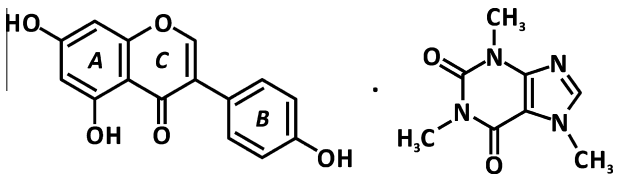
Genistein (>98% HPLC) was obtained from Sino-Future Bio-Tech Co. Ltd.; caffeine (98.5%) was obtained from Acros Organics and both were used without further purification. Pure grade solvents were purchased from Avantor Performance Materials Poland S.A. and used as received.

Cocrystallization via solvent-drop grinding

50.0 mg (0.186 mmol) of genistein and a 1:1 stoichiometric amount of caffeine (35.8 mg, 0.186 mmol) were combined along with solvent (one drop, ca. 25 μl) in a 5 mL stainless steel grinding jar with two 7 mm stainless steel grinding balls. Samples were ground in a Narva Vibrator Mill for 30 min (3 × 10 min with 5 min cooling periods) at a rate of 50 Hz, with addition of methanol, ethanol, isopropanol, acetone, ethyl acetate, acetonitrile, water or chloroform. Resulting solids were dried overnight at ambient conditions and characterized using Raman spectroscopy and X-ray powder diffraction (XRPD).

Cocrystallization via slow evaporation

Genistein (25.0 mg, 0.093 mmol) and caffeine (17.9 mg, 0.093 mmol) were combined in 7 mL of ethyl alcohol with stirring. Resulting solution was filtered and allowed to slowly evaporate at ambient temperature. After 4 days, plate-like crystals were harvested and used for single-crystal X-ray diffraction. The solid phase was filtered, washed with ethyl acetate, allowed to dry at ambient conditions and subjected to powder diffraction and thermal analysis (TG–DTA).



Scheme 1.

Slurry cocrystallization

Bulk synthesis for dissolution studies was performed in a slurry experiment. A stoichiometric amount of starting materials (2000.0 mg (7.253 mmol) of genistein and 1435.8 mg (7.253 mmol) of caffeine) was suspended in 20 mL of methanol and stirred vigorously on a stir plate, over a period of 24 h. Resulting material was filtered and allowed to dry at ambient temperature. Identity and purity of the cocrystal was analysed by means of XRPD. Alternatively, methanol can be replaced by ethyl acetate in a similar procedure.

FT-Raman spectroscopy

Raman spectra were collected on a MultiRAM FT-Raman spectrometer (Bruker Optik GmbH, Ettlingen, Germany). Samples were scanned in a range of 3600–50 cm^{-1} with 2 cm^{-1} resolution and using a 1064 nm laser light.

Powder diffraction analysis

X-ray powder diffraction (XRPD) analyses were carried out on a Bruker D8-Advance diffractometer equipped with a VÅNTEC-1 detector ($\lambda_{Cu\ K\alpha1}$ = 1.5406 Å). The equipment was operated at 30 kV and 40 mA, and data were collected at room temperature in the range of 2θ = 3–40°.

Single-crystal X-ray diffraction analysis

Crystallographic measurement was performed on a Xcalibur R automated four-circle diffractometer with graphite monochromatized Mo $K\alpha$ radiation at 100(2) K, using an Oxford Cryosystems cooler. Selected crystallographic data are provided in Table 1. Data collection, cell refinement, and data reduction and analysis were carried out with CRYSALISCCD and CRYSALISRED, respectively

Table 1
Selected crystallographic data, data collection and structure refinement details for **GenCaf**.

	GenCaf
CCDC No.	985174
Formula	$C_{15}H_{10}O_5{\cdot}C_8H_{10}N_4O_2$
M_r	464.43
Crystal system	Monoclinic
Space group (no.)	$P2_1/c$ (14)
Temperature (K)	100(2)
a, b, c (Å)	8.455(3), 18.668(6), 13.272(4)
β (°)	108.64(3)
V (Å^3)	1984.9(12)
Z	4
ρ_{calc} (g cm^{-3})	1.554
Radiation type	Mo $K\alpha$
μ (mm^{-1})	0.12
Crystal size (mm)	0.27 × 0.17 × 0.04
Reflns. collected	23,160
Reflns. independent	4647
Significant [$I > 2\sigma(I)$] reflns.	3309
R_{int}	0.072
$R[F^2 > 2\sigma(F^2)]$, $wR(F^2)$, S	0.074, 0.164, 1.11
Parameters refined	312
$\Delta\rho_{max}$, $\Delta\rho_{min}$(e Å^{-3})	0.38, −0.31