

A NON-CENTROSYMMETRIC POLYMORPH OF 5-HYDROXY-7-METHOXY-2-PHENYLCHROMAN-4-ONE

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ABSTRACT

The bioactive compound 5-hydroxy-7-methoxy-2-phenylchroman-4-one (pinostrobin, **I**), was isolated from aerial parts of *Nolana ramossissima* I.M. Johnst., a Chilean endemic species, using a combination of medium pressure column chromatography and high speed countercurrent liquid-liquid chromatography (HSCCC). The compound crystallized as a non-centrosymmetric polymorph in the orthorhombic chiral space group P2₁2₁2₁. Its structure had been previously reported by Shoja (*Acta Cryst.* C45, 828, 1989) and Yamovoi *et al.* (*Khim. Priir. Soedin* (5), 361, 2001) in the centrosymmetric space groups Pbc_a and P2₁/c, respectively.

INTRODUCTION

High speed countercurrent chromatography (HSCCC) is a support-free liquid-liquid partition chromatographic technique which eliminates the problem of the adsorption of a sample onto a solid support and has been widely used for the purification of natural products, including flavanones¹. In this work this fast technique has been applied in combination with medium pressure column chromatography for the purification of the phenolic constituents in an organic extract of aerial parts of *Nolana ramossissima* I.M. Johnst. (Solanaceae), an endemic species of Pajoso Valley, Northern Chile.

The main compound isolated, 5-hydroxy-7-methoxy-2-phenylchroman-4-one, synonym: pinostrobin is an important biologically active flavanone previously isolated from different plant materials. This flavanone is a constituent of *Pinus strobus* and many other pine species and, *inter alia*, *Artemisia campestris*, *Boesenbergia pandurata*, *Boesenbergia rotunda*, *Sarcandra glabra*, and *Polygonum lapathifolium*, and has been identified also in honey², propolis³, and trees frequently visited by bees such as *Alnus spp.* and *Populus spp.* Pinostrobin has shown antileukemic⁴, antioxidant⁵ and antibacterial activity³ and inhibits TNF- α and IL-1 β production⁶. Pinostrobin is an inducer of mammalian phase 2 chemoprotective and antioxidant enzymes² and an inhibitor of DNA topoisomerase I activity⁷. In our hands the title compound crystallized as a new non-centrosymmetric polymorph. The existence of polymorphic forms provides a unique opportunity for the investigation of structure-property relationships, since by definition the only variable among polymorphs is that of crystal structure, and one of the most effective strategies for studying structure-property relationships has been to follow the behavior of a physical property through a polymorphic phase change.

EXPERIMENTAL

General

Nolana ramossissima was collected in Pajoso, Antofagasta, Chile in September 2011. A voucher herbarium specimen is deposited in the laboratory of Natural Products, University of Antofagasta, with the number Nr 110403. Dried and finely powdered aerial parts of *N. ramossissima* (1562 g) were extracted with petroleum ether (2 L, 3 times in the dark, 24 h each time). After evaporation of the solvent *in vacuo* at 35 °C, a dark gummy extract (35 g) was obtained. The extract (20 g) was submitted to medium pressure column chromatography (Silica Gel 60 H, 7 x 40 cm) using n-hexane:ethyl acetate 9:1 as solvent system and 41 fractions of 50 mL each were collected. The fractions were combined according to their TLC analysis and 6 combined fractions were obtained. Fraction 4 (483 mg) was re-chromatographed using a Quattro MK7 HSCCC instrument⁸ equipped with three preparative stainless steel coils of 115 mL each (2.10 mm I.D.) and one SSI model Series II HPLC pump, using n-hexane:ethyl acetate:acetonitrile 5:3:5 v/v/v as solvent system. The filtered sample solution (5 mL) was introduced into the preparative (115 mL) column through a manual low-pressure sample injection valve using a 5 mL sample loop. The lower phase was used as the mobile phase and was pumped at a flow rate of 4.0 mL/min in the 'head-to-tail' mode. After the mobile phase front

emerged and hydrodynamic equilibrium was established in the column, the percentage of the retention of the stationary phase was recorded. Fractions of about 1.0 mL were collected using a Gilson fraction collector. The spin rate of the planetary coils was set at 850 rpm and the percentage of retention of the upper organic stationary phase was 72 % at 25 °C. From fractions 8-15, compound **I** (5-hydroxy-7-methoxy-flavanone, 120 mg) was obtained after recrystallization from ethyl acetate:

Colourless crystals, m.p. 96-97 °C. The molecular weight was determined by ESI-MS/MS with a mass spectrometer (HCT-Ultra ETD II, Bruker Daltonics, Germany) [M-H]⁻: 269.27, [M+H]⁺: 271, MS²: 241.9, 178.3, 161.8, 153.2. ¹H NMR (300 MHz, CDCl₃) δ ppm: 5.44 (1H, dd, *J* = 3.1 and 16.1 Hz), 3.80 (s, OCH₃), 3.12 (1H, dd, *J* = 3.3 and 12.9 Hz), 2.84 (1H, dd, *J* = 10.2 and 14.7 Hz), 6.10 (1H, d, *J* = 2.3 Hz), 6.12 (1H, d, *J* = 2.3 Hz), 7.47 (5H, m), 12.11 (1H, s, OH). ¹³C NMR (300 MHz, CDCl₃) δ ppm: 79.14 (C-2), 42.75 (C-3), 196.16 (C-4), 162.44 (C-5), 94.62 (C-6), 167.94 (C-7), 94.13 (C-8), 163.87 (C-9), 102.77 (C-10), 137.86 (C-1), 126.96 (C-2), 129.16 (C-3), 126.96 (C-4), 129.16 (C-5), 126.96 (C-6). ¹H and ¹³C NMR (Bruker Avance 300 MHz, CDCl₃). These data, together with ESI-MS/MS and correlations observed in the HSQC and HMBC spectra, are consistent with the structure of 5-hydroxy-7-methoxy-2-phenylchroman-4-one (pinostrobin, Figure 1) confirmed by comparison of spectroscopic data with those reported in the literature^{4,9}.

Crystal structure determination

Colorless block crystals of pinostrobin of approximate dimensions 0.1x0.08x0.4 mm were obtained by slow evaporation of a MeOH solution. A well-shaped and suitably-sized single crystal was selected for the X-ray diffraction structure determination experiment. The X-ray intensity data were collected at 293 K on an Enraf Nonius CAD4-MACH3 diffractometer, using MoK α graphite monochromatic radiation (λ = 0.71073 Å). CAD4 software was used for data collection and cell refinement, and HELENA software of the WINGX program was used for data reduction. The structure was solved by direct methods using the SHELXS2013 program¹² and the refinement was carried out using SHELXL 2013¹². All non-hydrogen atoms of the molecule were clearly solved and full-matrix least-square refinement of these atoms with anisotropic thermal parameters was carried out. Hydrogen atoms on carbon and oxygen atom were positioned stereochemically and were refined with fixed individual displacement parameters [U_{iso}(H) = 1.5U_{eq}(C or O) for methyl and hydroxyl groups or 1.2U_{eq}(C) for methine and methylene groups and the aromatic ring hydrogen atom] using a riding and rotating group model (for methyl and hydroxyl groups), with C-H bond lengths of 0.96, 0.97, 0.98 and 0.82 Å for methyl, methine, methylene and hydroxyl groups, respectively. The hydrogen atom of the hydroxyl group was located by difference Fourier synthesis and was kept rigid together with its respective oxygen atom during the subsequent refinements. WINGX software was used to analyze and prepare the data for publication¹³. Molecular graphics were prepared using OLEX2¹⁴ and Mercury¹⁶. Since the most electron-rich atom is the oxygen atom, which does not have a sufficiently large anomalous scattering (using MoK α radiation), it was not possible to determine the enantiomer present using the Flack method¹⁷. Table 1 summarizes the results.

Table 1. Crystallographic data, details of data collection and structure refinement parameters for compound (II).

Crystal data	
C ₁₆ H ₁₄ O ₄	V = 1311.9(5) Å ³
M _r = 270.27	Z = 4
Orthorhombic, P2 ₁ 2 ₁ 2 ₁ (N°19)	MoK α radiation
a = 9.1292(18) Å	μ = 0.098 mm ⁻¹
b = 9.5946(19) Å	T = 293(2) K
c = 14.977(3) Å	colourless, block, 0.1 x 0.08 x 0.4
Density (calculated)/Mg.m ⁻³ 1.368	F(000) 568
Data collection	
Enraf Nonius CAD4-MACH3 diffractometer	1305 reflections with I > 2 σ (I)
2456 measured reflections	R _{int} = 0.055
1752 independent reflections	
Theta range for data collection (θ)	2.521 to 25.948°
Index ranges	-2 ≤ h ≤ 11, -11 ≤ k ≤ 11, -18 ≤ l ≤ 18
Refinement	
R[(F ² > 2 σ (F ²)) = 0.035	183 parameters
wR(F ²) = 0.090	Flack's parameter = 0.000
S = 1.05	$\Delta\rho_{\max}$ = 0.25 eÅ ⁻³
Extinction coefficient 0.040(4)	$\Delta\rho_{\min}$ = -0.25 eÅ ⁻³

RESULTS AND DISCUSSION

The title compound (**I**) is a new polymorph of structures **II** and **III** reported by Shoja¹⁰ and by Yamovoi *et al.*¹¹. In the Shoja report, the compound crystallized in the orthorhombic space group Pbc₂ Z = 8, cell parameters a = 10.127(2) Å, b = 15.270(3) Å, c = 8.226(2) Å, V = 1270.9(5) Å³, whereas the structure reported by Yamovoi *et al.* is in the monoclinic space group P2₁/c, Z = 4, a = 10.172(2) Å, b = 16.079(2) Å, c = 8.079(3) Å, β = 91.74(1)°, V = 1320.756 Å³. In our case compound **I** crystallized in the orthorhombic chiral space group P2₁2₁2₁, Z = 4, a = 9.1292(18) Å, b = 9.5946(19) Å, c = 14.977(3) Å, V = 1311.9(5) Å³. In all three polymorphic forms, the tetrahydropyran ring has skew-boat conformation ($Q_1 = 0.455(6)$ Å, $\theta = 123.1(7)^\circ$, $\varphi = 249.0(8)^\circ$ mean, for three polymorphs)¹⁵ and the phenyl ring has an equatorial α -orientation relative to the tetrahydropyran ring, Figure 1. In Figure 2 structures **I** and **II** are superimposed in capped stick fashion. The overlay of molecular backbones clearly shows the conformational similarity between homologous atoms, except for the hydroxyl hydrogen atoms of the hydroxyphenyl group, where the H atoms point up and down in **I** and **II**, respectively. Another important difference between both structures is the orientation of the phenyl ring with respect to the plane defined by tetrahydropyran ring atoms O1-C8-C9-C10-C15 (RMSD = 0.043 Å and 0.018 Å for **I** and **II**, respectively), 85.56(15)° vs. 109.4(3)°. Similarly, the main difference between **I** and **III** is the orientation of the phenyl ring with respect to the plane defined by O1-C8-C9-C10-C15 (RMSD = 0.043 Å and 0.015 Å for **I** and **III**, respectively), 85.56(15)° vs. 38.5(3)°, Figure 3. The bond angles and distances are normal and similar to those in structures **II** and **III**, Table 2. In the three polymorphs the molecular conformation is stabilized by one intramolecular hydrogen bond between the carbonyl O atom and the H atom of the hydroxyl group with graph-set motif S(6)¹⁸, but the geometry of this interaction is different for each polymorph (H2O2...O4 = 1.893(2) Å, O2...O4 = 2.618(3) Å, O2-H2...O4 = 146.84(18)° for **I**; H2O2...O4 = 1.598(5) Å, O2...O4 = 2.574(7) Å, O2-H2...O4 = 174.2(4)° for **II**; H2O2...O4 = 1.79(6) Å, O2...O4 = 2.685(7) Å, O2-H2...O4 = 160(6)° for **III**).

CONCLUSIONS

The main conclusion of this work is that a new, non-centrosymmetric polymorph of 5-hydroxy-7-methoxy-2-phenylchroman-4-one (pinostrobin), was determined by X-ray methods, was isolated from an organic extract of aerial parts of *Nolana ramossissima* Johnst., an endemic species of the Paposo Valley, Northern Chile, using high speed countercurrent chromatography (HSCCC) and slow evaporation of a methanol solution.

Supplementary material

CCDC-1019211 contains the supplementary crystallographic data for this paper. These data can be obtained free of charge from the Cambridge Crystallographic Data Centre via www.ccdc.cam.ac.uk/data_request/cif.

Table 2. Selected bond distances (Å) and angles (°) for polymorphs **I**, **II** and **III**.

	I	II	III
C9-O4	1.238(4)	1.250(8)	1.232(8)
C11-O2	1.348(4)	1.367(8)	1.348(8)
C7-O1	1.448(3)	1.450(8)	1.441(7)
C15-O1	1.371(3)	1.369(7)	1.400(7)
C13-O3	1.360(3)	1.363(8)	1.406(7)
O3-C16	1.426(4)	1.416(9)	1.400(8)
C7-C1	1.448(3)	1.450(8)	1.441(7)
Angles			
C15-O1-C7	115.3(2)	114.1(5)	119.1(5)
C13-O3-C16	117.8(2)	119.3(6)	116.4(5)
C10-C11-O2	120.2(3)	119.2(6)	119.9(5)

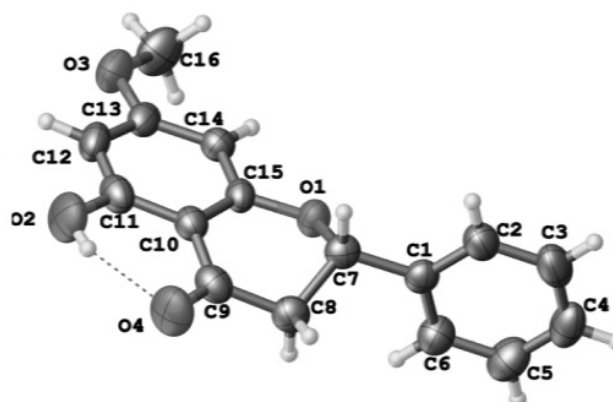
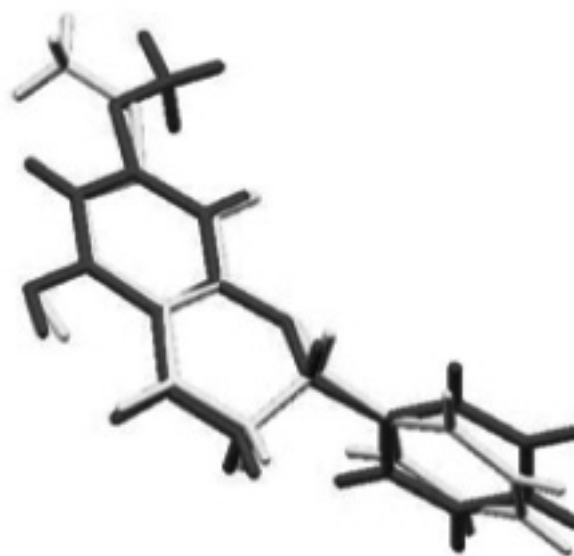
**Figure 1.** Molecular structure of **I** with the atom numbering scheme. The thermal ellipsoids are drawn at 40% probability level.**Figure 2:** Superimposed structures for the title compound (red) and polymorph **II** (Yellow).



Figure 3: Superimposed structures for the title compound (red) and polymorph III (blue).

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