Chemistry of Natural Compounds, Vol. 38, No. 6, 2002

SYNTHESIS AND STRUCTURE OF PINOSTROBIN OXIME AND ITS BIOLOGICAL ACTIVITY

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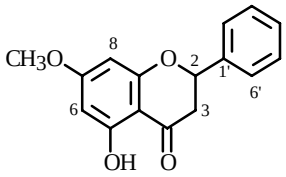
UDC 547.972:548.737

Reaction of pinostrobin with hydroxylamine and hydrazine formed the corresponding oxime and hydrazone with retention of the γ-pyrone ring. The structures of the modified pinostrobin derivatives were established using PMR, ^{13}C NMR, mass spectrometry, and X-ray analysis of pinostrobin oxime. Experiments in vivo revealed high hepatoprotective activity for pinostrobin oxime.

Key words: flavonoids, pinostrobin, reaction with hydroxylamine and hydrazine, X-ray analysis, hepatoprotective activity.

The literature contains little data on the chemical modification of flavonoids. Promising directions for the transformation of these compounds include the synthesis of nitrogeneous heterocycles [1-3]. The simplest method for achieving this goal is the recyclization of the γ-pyrone ring in reactions of flavones with hydroxylamine and hydrazone to form 5-(2-hydroxyphenyl)isoxazoles. Therefore, we studied these reactions for flavonones, in particular, pinostrobin.

Reaction of pinostrobin (**1**) with hydroxylamine hydrochloride in ethanol produced pinostrobin oxime (**2**) in 84% yield. The IR spectrum of **2** contains absorption bands at 1647 cm^{-1} due to C=N vibrations and at 1617 and 1578 cm^{-1}, corresponding to C=C stretches of the aromatic ring. The UV spectrum exhibits absorption bands at 205, 251, and 279 nm. The base peak in the mass spectrum corresponds to the molecular ion with *m*/*z* 285. The proton signals in the PMR of **2** differ little compared with those in starting **1** (Table 1).



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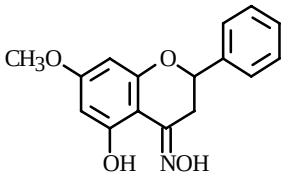

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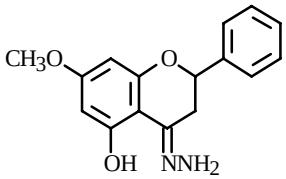

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Carrying out the reaction of **1** with hydroxylamine hydrochloride in pyridine, as described previously [1], formed a product identical with an authentic sample of **2**.

Reaction of **1** with hydrazine hydrate produced the corresponding hydrazone **3** in 72% yield. The IR spectrum of **3** exhibits absorption bands at 1630 cm^{-1} due to C=N vibrations and at 1591 cm^{-1}, corresponding to C=C stretches of the aromatic ring. The UV spectrum contains absorption bands at 210, 226, and 307 nm. The base peak in the mass spectrum corresponds to the molecular ion with *m*/*z* 284. The PMR (Table 1) spectrum differs little from that of starting **1** with the exception of the signals for H-2 and H-3, which are shifted relative to the signals of these protons in starting **1**. Protons H-6 and H-8 appear as doublets with spin—spin coupling constants 2.5 Hz at 6.07 and 6.05 ppm. This indicates that they are nonequivalent and that the γ-pyrone ring is retained.

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0009-3130/02/3806-0527$27.00 ©2002 Plenum Publishing Corporation

TABLE 1. PMR Spectra of Pinostrobin and Its Derivatives (δ, ppm, J/Hz), 500 MHz, Acetone-d_6

Compound	H-2	H-3	H-6	H-8	H-2′-H-6′	Other protons
1	5.58 dd (13.0, 3.0)	3.18 dd (17.0, 13.0) 2.83 dd (17.0, 3.0)	6.04 d (2.0)	6.08 d (2.0)	7.56 m (2H, H-2′, H-6′), 7.44 m (2H, H-3′, H-5′), 7.40 m	3.85 s (3H, OMe)
2*	5.18 1H, dd, (11.0, 3.0)	3.55, 1H, dd, (16.0, 3.0, H-3a) 2.80, 1H, dd, (16.0, 11.0, H-3b)	6.07, 2H, br.s		7.40 (3H, m, H-3′, H-4′, H-5′) 7.55 (2H, m, H-2′, H-6′)	3.75 3H, s, OMe
3	5.24 1H, dd, (12.0, 3.0)	3.70, 1H, dd, (17.0, 3.0, H-3a) 2.87, 1H, dd (17.0, 12.0, H-3b)	6.07 1H, d, (2.5)	6.05 1H, d, (2.5)	7.36 (1H, m, H-4′), 7.42 (2H, m, H-3′, H-5′) 7.53 (2H, m, H-2′, H-6′)	3.78 3H, s, OMe

*200 MHz, acetone-d_6.

TABLE 2. Bond Lengths (d, Å) in **2**

Bond	d	Bond	d
O1 C9	1.381(3)	C5 C10	1.404(3)
O1 C2	1.460(3)	C6 C7	1.378(3)
O2 N1	1.409(3)	C7 C8	1.398(3)
O3 C5	1.358(3)	C8 C9	1.364(3)
O4 C7	1.364(3)	C9 C10	1.414(3)
O4 C11	1.437(3)	C1′ C2′	1.374(4)
N1 C4	1.292(3)	C1′ C6′	1.396(4)
C2 C1′	1.503(3)	C2′ C3′	1.370(4)
C2 C3	1.517(3)	C3′ C4′	1.368(4)
C3 C4	1.491(3)	C4′ C5′	1.369(4)
C4 C10	1.459(3)	C5′ C6′	1.386(4)
C5 C6	1.383(3)		

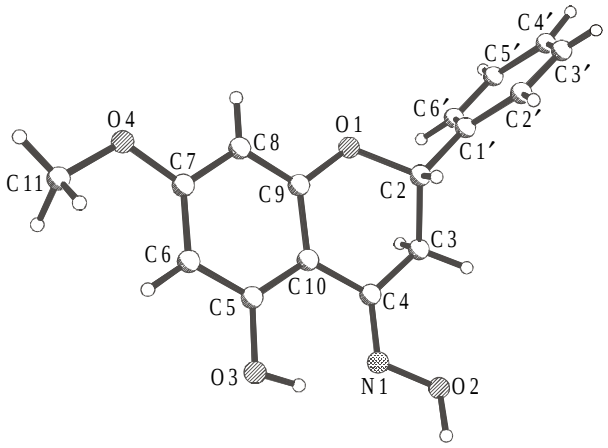

Fig. 1. Structure of pinostrobin oxime.

The structure of the product of reaction of pinostrobin and hydroxylamine was conclusively proved by an X-ray structure analysis.

Figure 1 shows a general view of **2**. The bond lengths (Table 2) and angles (Table 3) are close to the usual values [4]. The exception is the C3C2C1 angle, 115.2(2)°, which is significantly distorted from the tetrahedral value.

TABLE 3. Bond Angles (ω, deg) in Pinostrobin Oxime

Angle	ω	Angle	ω
C9 O1 C2	113.9(2)	C6 C7 C8	120.8(2)
C7 O4 C11	117.4(2)	C9 C8 C7	119.3(2)
C4 N1 O2	112.7(2)	C8 C9 O1	117.2(2)
O1 C2 C1′	107.7(2)	C8 C9 C10	122.3(2)
O1 C2 C3	109.1(2)	O1 C9 C10	120.5(2)
C1′ C2 C3	115.2(2)	C5 C10 C9	116.4(2)
C4 C3 C2	109.7(2)	C5 C10 C4	123.7(2)
N1 C4 C10	117.4(2)	C9 C10 C4	119.9(2)
N1 C4 C3	125.4(2)	C2′ C1′ C6′	118.5(2)
C10 C4 C3	117.2(2)	C2′ C1′ C2	119.5(2)
O3 C5 C6	116.7(2)	C6′ C1′ C2	122.0(2)
O3 C5 C10	121.2(2)	C3′ C2′ C1′	120.8(3)
C6 C5 C10	122.1(2)	C4′ C3′ C2′	120.8(3)
C7 C6 C5	119.2(2)	C3′ C4′ C5′	119.7(3)
O4 C7 C6	123.8(2)	C4′ C5′ C6′	120.1(3)
O4 C7 C8	115.4(2)	C5′ C6′ C1′	120.2(3)

TABLE 4. Atomic Coordinates (Å×10^4; for H, Å×10^3) in Cell Fractions for Pinostrobin Oxime

Atom	x	y	z	Atom	x	y	z
O1	3547(1)	1160(2)	1176(1)	C4′	2508(3)	-643(4)	-2268(2)
O2	3780(2)	7441(2)	596(1)	C5′	3508(3)	-362(4)	-1818(2)
O3	4018(2)	6110(2)	3361(2)	C6′	3694(2)	602(4)	-959(2)
O4	3787(1)	345(2)	4567(1)	H02	365(3)	863(6)	77(3)
N1	3828(2)	6502(3)	1480(2)	H03	397(3)	662(5)	276(2)
C2	3028(2)	2293(3)	400(2)	H2	230(2)	265(4)	60(2)
C3	3660(2)	3949(3)	370(2)	H3A	332(2)	470(3)	-17(2)
C4	3772(2)	4837(3)	1343(2)	H3B	438(3)	352(4)	23(2)
C5	3908(2)	4378(3)	3173(2)	H6	401(2)	377(3)	463(2)
C6	3925(2)	3291(3)	3976(2)	H8	363(2)	-40(3)	277(2)
C7	3814(2)	1520(3)	3829(2)	H11A	382(2)	-1(4)	595(2)
C8	3695(2)	819(3)	2882(2)	H11B	454(2)	175(4)	575(2)
C9	3680(2)	1909(3)	2098(2)	H11C	322(2)	188(4)	557(2)
C10	3789(2)	3734(3)	2208(2)	H2′	129(2)	145(4)	-77(2)
C11	3862(3)	1016(4)	5550(2)	H3′	98(2)	-19(4)	-219(2)
C1′	2864(2)	1258(3)	-537(2)	H4′	239(2)	141(4)	-285(2)
C2′	1869(2)	960(4)	-1004(2)	H5′	407(3)	-87(4)	-207(2)
C3′	1695(3)	27(4)	-1862(2)	H6′	437(2)	80(4)	-68(2)

The six-membered ring is planar within ±0.002 Å. The heterocycle adopts a distorted 2β-boat conformation (ΔC_S^2 = 1.4°). Atoms C3, C4, C10, C9, and O1 are coplanar within ±0.01 Å whereas C2 deviates from this plane to the β-side by 0.68 Å.

Phenyl ring B has an equatorial α-orientation relative to ring C and is situated perpendicular to its average plane. Torsion angle H2C2C1′C6′ is 1.9°. The structure of **2** has an intramolecular H-bond O3–H...N1 (distance O...N 2.58 Å, H...N 1.75 Å, angle O3H03N1 151.1°).

It is noteworthy that the methoxy and phenyl ring in the structures of **1**, which we studied previously, and **2** have different orientations [5]. Thus, torsion angle C11O4C7C8 is 177.4° and close to the value (176.9°) corresponding to one of the polymorphs of **1** [6]. Angle H2C2C1′C6′ is 1.9° and close to the value (4.6°) corresponding to pinostrobin acetate [7].

Pinostrobin and its oxime influence positively to different extents the course of experimental hepatitis. In all three

experimental groups the ALT and AST activity decreases. The ALT activity on the 7th day after the start of CCl_4 administration in groups receiving **2** and **1** was less than in untreated animals by 31 and 7.3%; AST, 26.5 and 25.8%. By the 21st day, the ALT and AST content in rats receiving **2** was practically normal. It exceeded the norm by 15-20% in those receiving **1** and *Salsola collina* extract.

The cholesterol content, serving as an indicator of cholestase syndrome, decreased progressively in all experimental groups over the course of the experiment.

The total blood protein content on the 7th day decreased compared with untreated rats in all studied groups including the control. The total protein level stabilized by the 21st day in groups receiving **2** and **1** and salsocollin as a standard. This indicates that the protein-synthesizing function of the liver had stabilized.

Thus, **2** exhibits distinct hepatoprotective properties and has a better therapeutic effect than **1** and *Salsola collina* extract. The hepatoprotective action of **2** is apparently due to its antioxidant and membrane-stabilizing properties.

EXPERIMENTAL

IR spectra were recorded on a Vector 22 instrument; UV spectra (in ethanol solution), on a Specord UV-VIS instrument. NMR spectra were recorded on a DRX-500 (Bruker) spectrometer (working frequency 500.13 MHz for ^1H and 125 MHz for ^{13}C) using standard Bruker programs. High-resolution mass spectra (EI, 70 eV) were obtained in a MAT 8200 (Finnigan) instrument.

Pinostrobin Oxime (2). Compound **1** (200 mg) was treated with hydroxylamine hydrochloride (77 mg) dissolved in ethanol. The mixture was treated with $NaHCO_3$ (100 mg). The reaction was carried out at 60°C. After 4 h, the mixture was treated with HCl (1%) and H_2O to give colorless rhombic crystals in 84% yield [8], mp 182-184°C (ethylacetate), $C_{16}H_{15}NO_4$.

IR spectrum (ν, cm^{-1}, KBr): 3732, 3647, 3431 (OH), 3008, 2918, 2848, 1647 (C=N), 1617, 1578 (C=C), 1515, 1458, 1443, 1389, 1339, 1276, 1227, 1209, 1192, 1149, 1094, 1068, 1054, 1029, 990, 964, 948, 922, 887, 866, 820, 777, 750, 737, 704.

UV spectrum (λ, nm, log ε, EtOH): 205 (4.37), 251 (3.61), 279 (4.13).

Mass spectrum [*m/z*, *I* (%)]: 285 $[M]^+$ (100.0), 268 (16.1), 266 (19.3), 208 (46.4), 179 (9.5), 164 (8.8), 136 (5.3), 105 (13.3), 104 (25.9), 91 (12.4), 77 (23.3), 69 (12.1), 39 (5.7).

Elemental analysis: Found (*m/z*): 285.10144.

Calculated for $C_{16}H_{15}NO_4$: *m/z* = 285.10010.

TLC: system 1, R_f 0.48 (petroleum ether:ethylacetate, 2:1).

^{13}C NMR spectrum (acetone-d_6, 50.324 MHz, δ, ppm): 55.69 q, 77.46 d, 94.76 d, 96.29 d, 127.16 d, 129.12 d, 129.39 d, 140.99 s, 154.48 s, 150.31 s, 160.69 s, 163.58 s.

Pinostrobin Hydrazone (3). Compound **1** (200 mg) was treated with $N_2H_4 \cdot H_2O$ solution (5 mL, 55%). The reaction mixture was left for 15 min and treated with $NaHCO_3$ solution (20%) and water. The organic layer was dried over Na_2SO_4. The solvent was removed in vacuum to produce white crystals in 72% yield, $C_{16}H_{18}N_2O_3$, mp 188-189°C (EtOH).

IR spectrum (ν, cm^{-1}, KBr): 3075 (CH arom.), 3036, 2996, 2967, 2935, 2849, 1630 (C=N), 1591 (C=C arom.), 1458, 1433, 1404, 1371, 1345, 1294, 1259, 1212, 1194, 1156, 1105, 1078, 1041, 1026, 1002, 964, 892, 825, 769, 745, 701, 651, 611, 584, 562.

UV spectrum (λ, nm, log ε, EtOH): 210 (4.46), 226 (4.43), 307 (4.23).

Mass spectrum [*m/z*, *I*(%)]: 284 $[M]^+$ (100), 268 (14.7), 266 (10.3), 253 (7.4), 247 (25.8), 238 (4.3), 233 (7.1), 220 (5.2), 211 (3.8), 206 (3.4), 193 (2.2), 192 (4.8), 191 (6.6), 177 (4.2), 173 (4.1), 166 (4.4), 165 (6.8), 160 (6.7), 159 (48.8), 145 (6.7), 144 (40.4), 131 (4.5), 118 (5.3), 115 (3.9), 104 (10.2), 103 (20.4), 91 (8.6), 77 (11.3), 69 (8.4), 56 (32.8), 42 (6.2), 28 (14.1).

^{13}C NMR (acetone-d_6, 125.76 MHz, δ, ppm): 166.1, 165.0, 163.4, 162.7, 160.8, 140.9, 129.5, 129.4, 127.2, 100.6, 96.1, 94.4, 78.1, 55.8, 33.1, 25.39.

X-ray Structure Analysis. Cell constants and intensities of 2389 independent reflections were measured on a Bruker P4 diffractometer (Mo Kα, graphite monochromator, θ/2θ-scanning, $2\theta < 50°$). Crystals are monoclinic, a = 12.995(1), b = 7.674(1), c = 13.774(1) Å, β = 98.71(1)°, V = 1357.8(2) $Å^3$, d_{calc} = 1.396, Z = 4 ($C_{16}H_{15}NO_4$), space group $P2_1/n$. The structure was solved by direct methods and refined by anisotropic full-matrix least-squares methods for nonhydrogen atoms.

Hydrogen atoms were found from a difference synthesis and refined isotropically. A total of 1579 reflections with $I > 2\sigma(I)$ were used in the calculations. Absorption corrections were made using ψ-curves. The final agreement factors are $R = 0.048$ and $R_w = 0.109$. The structure was solved using the SHELX-86 program and refined using SHELXL-97. Table 4 lists the atomic coordinates.

Hepatoprotective Activity. Experimental hepatitis was induced in animals by subcutaneous administration of CCl_4 in oil (50%, 0.4 mL/100 g mass) once per day for four days. From the third day and until the end of the experiment, animals were treated daily with the tested compounds (**1** and **2**) at a dose of 25 mg/kg (intraperitoneally through a probe). Control animals received distilled water of the corresponding volumes. The standard was an alcohol extract of *Salsola collina* at a dose of 100 mg/kg, which is a hepatoprotective preparation of salsocollin [9]. Untreated animals (not receiving CCl_4) were kept under analogous conditions.

Biochemical blood analysis (total protein, ALT, AST, cholesterol, bilirubin) on the 7th, 14th, and 21st days of the experiment provided an indicator of the course of the experimental hepatitis and the hepatoprotective activity of the tested preparations. Transaminase activity was determined by the S. Reitman—S. Frankel method; total protein, by the biuret method; cholesterol level, by reflotron using indicator bands [10].

Furthermore, we observed the general conditions of the animals: motor activity, appetite, reaction to external stimuli. Results were treated statistically by variational analysis with a Student estimate of the significance of the differences [11].

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