PIRONETIN†, A NOVEL PLANT GROWTH REGULATOR PRODUCED BY *Streptomyces* sp. NK10958

II. STRUCTURAL ELUCIDATION

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A novel plant growth regulator, pironetin was isolated from the culture broth of *Streptomyces* sp. NK10958. The structure of pironetin was determined to be (5*R*,6*R*)-5-ethyl-5,6-dihydro-6-[(*E*)-(2*R*,3*S*,4*R*,5*S*)-2-hydroxy-4-methoxy-3,5-dimethyl-7-nonenyl]-2H-pyran-2-one by FAB-MS, ^{1}H and ^{13}C NMR, COSY, COLOC, DEPT, IR, X-ray crystallographic analyses and adapted MOSHER's method.

Pironetin (Fig. 1) is a new microbial secondary metabolite having the activity of shortening of plant height. The taxonomy, production, isolation and preliminary characterization of pironetin were reported previously[1]. In this paper we describe the structural elucidation.

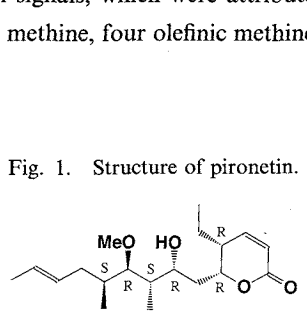
Results and Discussion

The IR spectrum of pironetin (Fig. 2) showed major absorptions at 3511, 2966, 1728 cm^{-1} indicating hydroxyl group, C-H bonds and α,β-unsaturated δ-lactone, respectively. The high resolution positive ion FAB-MS of pironetin was recorded as 325.2386, which correspond to $C_{19}H_{33}O_4$ (calcd 325.2379; M+H).

The ^{1}H NMR and ^{13}C NMR spectra of pironetin are shown in Figs. 3 and 4, respectively. Chemical shifts and DEPT data in the NMR spectra are shown in Table 1. The ^{13}C NMR spectrum, ^{13}C-^{1}H COSY and DEPT experiments revealed the presence of nineteen carbon signals, which were attributed to four methyl, one methyloxy, three methylene, three methine, three oxy methine, four olefinic methine, and one carbonyl carbons.

Pironetin has one hydroxyl residue (δ 3.45) at C-7 (δ 67.3) oxy methine carbon because one acetyl group has been derived by the acetylation with Ac_2O in pyridine according to NMR and IR data (data not shown).

Fig. 1. Structure of pironetin.



The ^{1}H-^{1}H COSY data showed the couplings

† Pironetin was originally called as NK10958.

Fig. 2. IR spectrum of pironetin (KBr).

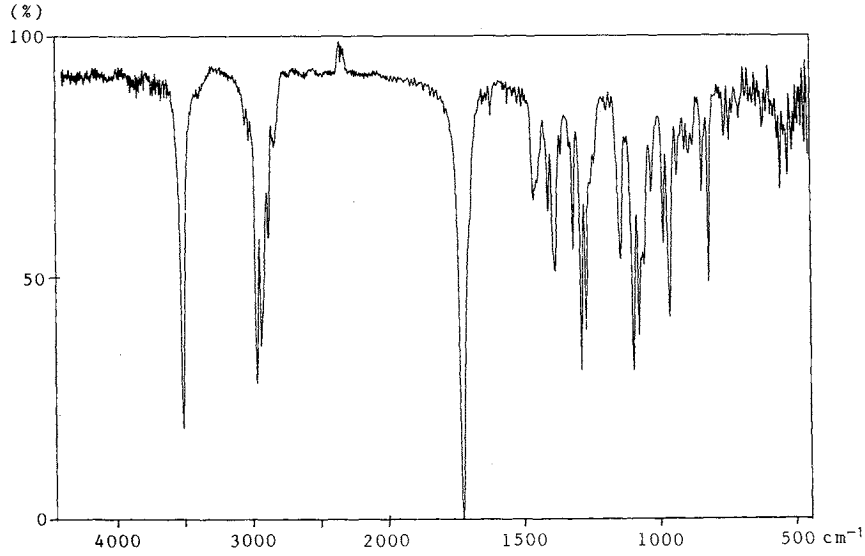


Fig. 3. ^{1}H NMR spectrum of pironetin (400 MHz in $CDCl_3$).

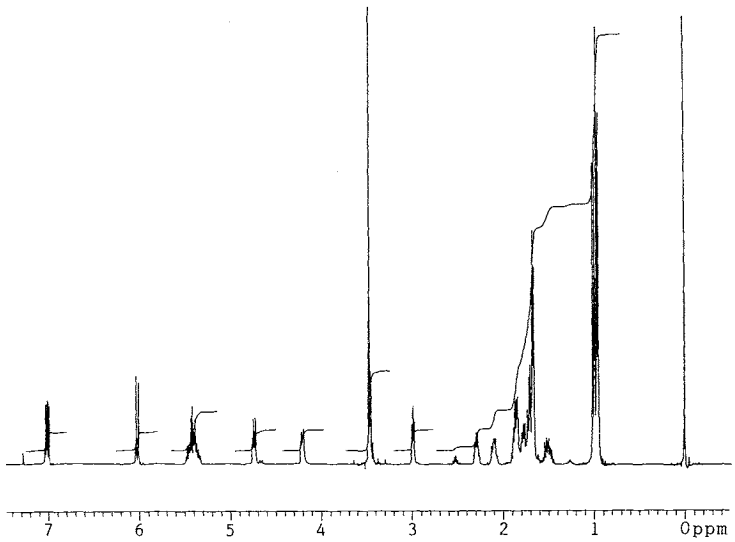
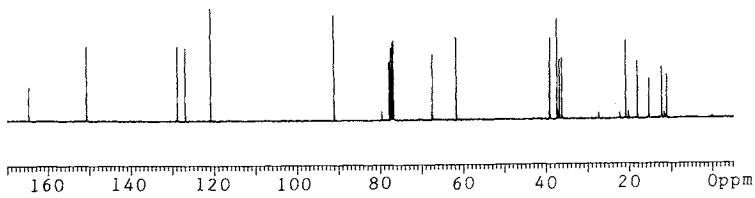


Fig. 4. ^{13}C NMR spectrum of pironetin (100 MHz in $CDCl_3$).



among 2-H and 3-H, 3-H and 4-H, 4-H and 15-H, 15-H and 16-H, 4-H and 5-H, 5-H and 6-H, 6-H and 7-H, 7-H and -OH, 8-H and 9-H, 8-H and 17-H, 9-H and 10-H, 10-H and 18-H, 11-H and 12-H, 12-H and 13-H, 13-H and 14-H, respectively. Partial structures A, B, C and D (Fig. 5) can be constructed from above NMR data. The COLOC data between 11-H and C-10, 11-H and C-18 indicated the connection of the partial structures A and B. The COLOC data between 7-H and C-17, 3-H and C-1 indicated the connections of the partial structures B and C, C and D, respectively. The unsaturation number of pironetin is 4 based on a calculation of molecular formula. There are two double bonds and one carbonyl residue, so the remaining unsaturation number is one. Partial structures C and D make one cyclic structure to satisfy the unsaturation number of pironetin. The IR spectrum of pironetin showed α,β-unsaturated δ-lactone (1728 cm^{-1}). The ^1H NMR signals at 6.03 ppm (2-H) and 7.02 ppm (3-H) are assigned to the α and β proton, respectively. The ^{13}C NMR chemical shifts of pironetin suggested that both C-1 (δ 164.7) and C-5 (δ 77.7) connect to oxygen in the six memberd lactone ring. The methyloxy residue was placed at C-9 oxy methine carbon according to the COLOC data. The coupling constants ($J_{2,3}=9.9$ Hz, $J_{12,13}=15.4$ Hz) showed the geometrical isomerisms are *Z* and *E*, respectively. Based on the above spectral analyses, the chemical structure of pironetin was proposed as shown in Fig. 5.

Table 1. ^{13}C and ^1H NMR chemical shift and DEPT data of pironetin.

Carbon number	Carbon type[a]	δ_C[b]	δ_H[c]
1	C=O	164.7	
2	CH=	120.7	6.03 (1H, d, J=9.9 Hz)
3	CH=	150.7	7.02 (1H, dd, J=9.9, 6.2 Hz)
4	CH	39.0	2.29 (1H, m)
5	CH–O	77.7	4.74 (1H, m)
6	CH_2	36.7	1.71 (2H, m)
7	CH–O	67.3	4.21 (1H, br d)
8	CH	38.9	1.77 (1H, m)
9	CH–O	91.0	2.99 (1H, dd, J=6.2, 4.4 Hz)
10	CH	36.1	1.85 (1H, m)
11	CH_2	37.2	1.85 (1H, m), 2.10 (1H, m)
12	CH=	128.7	5.37 (1H, dt, J=15.4, 5.9 Hz)
13	CH=	126.9	5.45 (1H, dq, J=15.4, 5.9 Hz)
14	CH_3	17.9	1.67 (3H, d, J=5.9 Hz)
15	CH_2	20.7	1.51 (1H, m), 1.71 (1H, m)
16	CH_3	11.0	0.97 (3H, t, J=7.3 Hz)
17	CH_3	12.1	1.00 (3H, d, J=7.3 Hz)
18	CH_3	15.2	0.97 (3H, d, J=7.3 Hz)
19	CH_3–O	61.6	3.47 (3H, s)
—	–OH		3.45 (1H, d, J=2.6 Hz)

[a] Based on ^{13}C DEPT NMR experiments.
[b] 100 MHz in $CDCl_3$ (ppm).
[c] 400 MHz in $CDCl_3$ (ppm).

Single crystal X-ray analysis was performed and confirmed the above structure, also it provided the complete structure including relative stereochemistry (Fig. 6). The final R factor was 0.078 for 1494

Fig. 5. Partial structures, ^1H-^1H COSY and COLOC coupling patterns of pironetin.

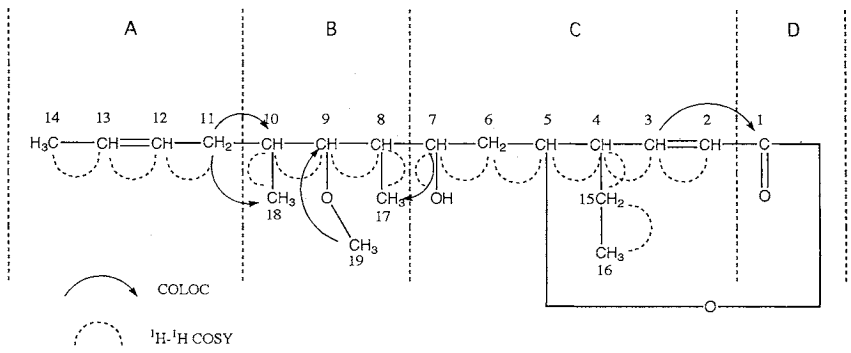
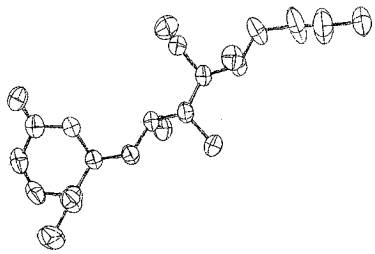


Fig. 6. A perspective view of pironetin.



observed reflections.

We used adapted MOSHER's method[2] to determine absolute stereochemistry of pironetin. The ^{1}H NMR properties of the (*R*)- and (*S*)-2-methoxy-2-phenyl-2-(trifluoromethyl)acetic acid (MTPA) esters of pironetin were listed in Table 2. A $\Delta\delta$ (ppm) is subtraction of the chemical shift of (*R*)-MTPA-ester from the chemical shift of (*S*)-MTPA-ester. According to MOSHER's method principle[2], the C-7 carbon has (*R*)-stereochemistry, therefore we determined all stereochemistry as *R* (C-4), *R* (C-5), *R* (C-7), *S* (C-8), *R* (C-9) and *S* (C-10) (Fig. 1).

Table 2. ^{1}H NMR chemical shift data of MTPA esters of pironetin.

No.	(*S*)-MTPA (ppm)	(*R*)-MTPA (ppm)	$\Delta\delta$ (ppm)
2	6.02	6.00	+0.02
3	7.00	6.96	+0.04
4	2.215	2.155	+0.06
5	4.39	4.32	+0.07
6	2.044	1.928	+0.116
6	2.135	2.041	+0.094
7	5.47	5.54	−0.07
8	1.754	1.767	−0.013
9	2.85	2.85	0.0
10	1.613	1.625	−0.012
12	5.359	5.372	−0.013
13	5.424	5.442	−0.018
14	1.65	1.66	−0.01
15	1.541	1.497	+0.044
15	1.795	1.726	+0.069
16	0.97	0.95	+0.02
17	0.83	0.84	−0.01
18	0.79	0.80	−0.01
19	3.39	3.41	−0.02

$\Delta\delta$ (ppm) = {(*S*)-MTPA (ppm)} − {(*R*)-MTPA (ppm)}.

The partial structure (4-ethyl-5-substituted-α,β-unsaturated-δ-lactone) of pironetin is involved in phosphazomycin C[3]. Phosphazomycin C has antifungal and antitumor activities but was not described with plant growth regulative activity. Other similar compound that has 4-methyl-5-substituted-α,β-unsaturated-δ-lactone is kazusamycin[4]. Kazusamycin also showed antitumor activity but not described with plant growth regulative activity. The cytotoxicity of pironetin to tumor cell lines will be reported separately.

Experimental

IR, FAB-MS, NMR and X-ray

IR spectrum was recorded on a Perkin Elmer model 1600 FT-IR. FAB-MS spectrum was obtained on JEOL JMS-AX505HA mass spectrometer. NMR spectra were recorded on JEOL GX-400 NMR spectrometer with ^{1}H NMR at 400 MHz and ^{13}C NMR at 100 MHz. X-ray reflections were measured on a Philips PW1100 diffractometer.

MTPA Esters of Pironetin

Commercially available (*R*)- and (*S*)-MTPA acids (Aldrich) were used without purification. (*R*)- and (*S*)-MTPA chlorides were prepared according to the literature[2]. (*R*)-MTPA chloride (5.1 mg, 20 μmol) was added to a solution of pironetin (3.3 mg, 10 μmol) in pyridine (20 μl) and the solution was allowed to stand at room temperature for 16 hours. The solution was poured into cold water, followed by extraction with EtOAc. The organic layer was washed by water and dried over $MgSO_4$. The residue obtained after evaporation of the solvent was chromatographed on Sephadex LH-20 column with MeOH. The MTPA ester was checked by using TLC and the fractions contained MTPA ester were collected and concentrated *in vacuo* to give pure (*R*)-MTPA ester (5 mg, 92 %). (*S*)-MTPA chloride (5.1 mg, 20 μmol) was added to a solution of pironetin (3.3 mg, 10 μmol) in pyridine (20 μl), and the solution was allowed to stand at room temperature for 17 hours. After purification of the same method mentioned above to give pure (*S*)-MTPA

ester (5 mg, 92%).

Acknowledgments

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