NK10958P, a Novel Plant Growth Regulator Produced by *Streptomyces* sp.

KOUICHI TSUCHIYA, SHINICHI KOBAYASHI, TAKAAKI NISHIKIORI, TAIZO NAKAGAWA and KUNIAKI TATSUTA†

Applied Microbiology Research Center, Nippon Kayaku Co., Ltd., 225-1, Koshikiya, Horigome, Ageo-shi, Saitama 362, Japan
†Graduate School of Science and Engineering, Advanced Research Institute for Science and Engineering, Waseda University, Ohkubo, Shinjuku, Tokyo 169, Japan

(Received for publication October 28, 1996)

We previously reported on the production of pironetin, a plant growth regulator, by *Streptomyces* sp. NK10958[1]. Additional isolation studies from the culture broth led to the discovery of another active compound named NK10958P, which is related to pironetin (Fig. 1). In this paper, we report the isolation, structural elucidation and biological properties of NK10958P.

The production of active compounds, NK10958P and pironetin, was carried out at 27°C for 65 hours using a 200-liter tank fermenter as previously described[1].

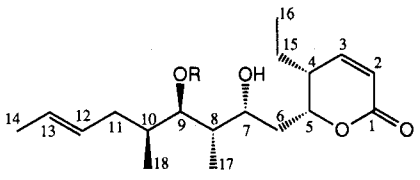
NK10958P was isolated from the culture broth by monitoring plant growth regulation. The culture broth (200 liters) of *Streptomyces* sp. NK10958 was separated into mycelia (30 kg) and filtrate (190 liters) by a filtration. The filtrate was applied on a column of Diaion HP-20 (Mitsubishi Chemical Ltd., 10 liters) washed with Me_2CO-H_2O (1:1, 20 liters). The active fraction was eluted with Me_2CO (20 liters) and evaporated under the reduced pressure to give aqueous solution (4 liters). This aqueous solution was extracted with EtOAc (3×4 liters) and then the EtOAc extract was dried over anhydrous $MgSO_4$ and concentrated *in vacuo* to a brown oil (54.0 g). The oily residue was applied on a silica gel column (Fuji Silysia Chemical Ltd., BW-350, 1 kg) and eluted with a stepwise gradient of *n*-hexane-Me_2CO. The first active fraction containing pironetin was eluted with *n*-hexane-Me_2CO (5:2), and the second active fraction containing NK10958P was eluted with *n*-hexane-Me_2CO (2:1).

The mycelial cake (30 kg) was extracted with MeOH (60 liters). The MeOH extract was concentrated *in vacuo* to give aqueous solution (8 liters). The resulting solution was extracted with EtOAc (3×8 liters) and then the EtOAc extract was dried over anhydrous $MgSO_4$ and concentrated *in vacuo* to a brown oil (68.6 g). This oily residue was applied on a silica gel column (Fuji Silysia Chemical Ltd., BW-350, 1.5 kg) and eluted with a stepwise gradient of *n*-hexane-Me_2CO as described above.

Pironetin (8.0 g) was isolated from the first active fractions using silica gel column chromatography[1]. The combined active fractions from silica gel columns were concentrated under the reduced pressure to give a crude oil (28.2 g). This crude oil was rechromatographed by a silica gel column (Fuji Silysia Chemical Ltd., BW-350, 500 g) with the same gradient system to yield oily residue (11.0 g). This oily residue was chromatographed on Sephadex LH-20 (Pharmacia, 900 ml, fraction size 11 g) with Me_2CO to give oily material (523 mg). Finally, NK10958P (377 mg) was crystallized from *n*-hexane as colorless needles.

The physico-chemical properties of NK10958P and pironetin are summarized in Table 1. The IR spectrum of NK10958P was very similar to that of pironetin[1]. The molecular formula of NK10958P was determined to be $C_{18}H_{30}O_4$ on the basis of HRFAB-MS measurements

Fig. 1. Structures of NK10958P and pironetin.



NK10958P : R = H

Pironetin : R = Me

Table 1. Physico-chemical properties of NK10958P and pironetin.

	NK10958P	Pironetin
Appearance	Colorless needle	Colorless needle
$[\alpha]_D^{20}$	−123.0° (*c* 1.0, $CHCl_3$)	−136.6° (*c* 1.0, $CHCl_3$)
MP	77~78°C	78~79°C
FAB-MS *m/z*	311 $(M+H)^+$, 333 $(M+Na)^+$	325 $(M+H)^+$, 347 $(M+Na)^+$
HR-MS Found	333.2027	325.2386
Calcd	333.2042 ($C_{18}H_{30}O_4Na$)	325.2379 ($C_{19}H_{33}O_4$)
Molecular formula	$C_{18}H_{30}O_4$ (MW: 310)	$C_{19}H_{32}O_4$ (MW: 324)
UV λ_{max}^{MeOH} nm ($E_{1cm}^{1\%}$)	End absorption	End absorption
IR ν_{max} cm^{-1}	3544, 2967, 1719, 964	3511, 2966, 1728, 964
TLC Rf value*	0.25	0.40

* Rf value: Silica gel TLC (Kiesergel 60F 0.25 mm, Merck) was used with developing solvent *n*-hexane-acetone (5:2).

Table 2. ^{13}C and ^{1}H NMR chemical shifts of NK10958P and pironetin in $CDCl_3$.

No.	NK10958P			Pironetin		
	δ C[a]	Carbon type[b]	δ H[c]	δ C[a]	Carbon type[b]	δ H[c]
1	165.0	C=O		164.7	C=O	
2	120.7	CH=	6.03 (1H, d, J = 9.5Hz)	120.7	CH=	6.03 (1H, d, J = 9.9Hz)
3	151.0	CH=	7.03 (1H, dd, J = 9.5, 6.1Hz)	150.7	CH=	7.02 (1H, dd, J = 9.9, 6.2Hz)
4	39.1	CH	2.30 (1H, m)	39.0	CH	2.29 (1H, m)
5	77.2	CH-O	4.80 (1H, dt, J = 10.1, 3.0Hz)	77.7	CH-O	4.74 (1H, m)
6	34.7	CH_2	1.72 (1H, m), 1.8 (1H, m)	36.7	CH_2	1.71 (2H, m)
7	77.4	CH-O	3.57 (1H, dd, J = 8.4, 3.3Hz)	67.3	CH-O	4.21 (1H, br d)
8	39.8	CH	1.86 (1H, m)	38.9	CH	1.77 (1H, m)
9	69.5	CH-O	4.17 (1H, bd, J = 10.6Hz)	91.0	CH-O	2.99 (1H, bd, J = 6.2, 4.4Hz)
10	35.3	CH	1.68 (1H, m)	36.1	CH	1.85 (1H, m)
11	37.5	CH_2	1.98 (1H, m), 2.08 (1H, m)	37.2	CH_2	1.85 (1H, m), 2.10 (1H, m)
12	129.4	CH=	5.43 (1H, dt, J = 15.3, 6.6Hz)	128.7	CH=	5.37 (1H, dt, J = 15.4, 5.9Hz)
13	126.9	CH=	5.49 (1H, dq, J = 15.3, 5.8Hz)	126.9	CH=	5.45 (1H, dq, J = 15.4, 5.9Hz)
14	18.0	CH_3	1.67 (3H, d, J = 5.8Hz)	17.9	CH_3	1.67 (3H, d, J = 5.9Hz)
15	20.8	CH_2	1.52 (1H, m), 1.70 (1H, m)	20.7	CH_2	1.51 (1H, m), 1.71 (1H, m)
16	11.0	CH_3	0.98 (3H, t, J = 7.5Hz)	11.0	CH_3	0.97 (3H, t, J = 7.3Hz)
17	12.2	CH_3	0.88 (3H, d, J = 7.0Hz)	12.1	CH_3	1.00 (3H, d, J = 7.3Hz)
18	12.2	CH_3	0.86 (3H, d, J = 7.0Hz)	15.2	CH_3	0.97 (3H, d, J = 7.3Hz)
19	—		—	61.6	CH_3-O	3.47 (3H, s)
7-OH			2.47 (1H, bs)			3.45 (1H, d, J = 2.6Hz)
9-OH			3.31 (1H, bs)			—

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

(*m/z* Found 333.2027, Calcd 333.2042 for $C_{18}H_{30}O_4Na$). This molecular formula had one less carbon atom and two less protons than that of pironetin. The ^{1}H NMR spectrum ($CDCl_3$) of NK10958P displayed 30 proton signals and its ^{13}C NMR spectrum ($CDCl_3$) showed 18 resolved peaks (Table 2), supporting the molecular formula. The carbon atoms in the molecule were classified into four methyl, three methylene, three methine, three oxy methine, four sp^2 methine and one carbonyl carbons by the analysis of the DEPT spectra. The proton assignments were made by the analyses of ^{1}H-^{1}H COSY and ^{13}C-^{1}H COSY. Structural elucidation of NK10958P was achieved by the detailed NMR spectral analyses and the comparison with those of pironetin. The methoxy proton and carbon signals corresponding to 19-CH_3 (δ_H 3.47, δ_C 61.6) of pironetin were not observed in the ^{1}H and ^{13}C NMR spectra of NK10958P. A hydroxyl proton signal was observed at δ_H 3.31 (9-OH) instead of 19-CH_3 signals. The carbon signal corresponding to C-9 (δ_C 69.5) of NK10968P resonated at higher field than that of pironetin (δ_C 91.0). Thus NK10958P was deduced to be 19-*O*-demethyl analogue of pironetin. The optical rotations of NK10958P and pironetin were $-123.0°$ and $-136.6°$, respectively (Table 1). It can therefore be presumed that the stereochemistry of NK10958P is consistent with pironetin. In the biosynthetic study of pironetin, the 19-CH_3 was derived from methyl of methionine[3]. Consequently, NK10958P was regarded as a precursor of pironetin.

NK10958P was tested for plant growth regulative activity against *Sorghum bicolor* in comparison with pironetin. NK10958P and pironetin showed 50% shortening of plant height at a dose of 109 ppm and 115 ppm, respectively.

Acknowledgments

We are thankful to Mr. M. SATO, Nippon Kayaku Co. Ltd., for NMR measurements.

References

1) KOBAYASHI, S.; K. TSUCHIYA, T. HARADA, M. NISHIDE, T. KUROKAWA, T. NAKAGAWA, N. SHIMADA & K. KOBAYASHI: Pironetin, a novel plant growth regulator produced by *Streptomyces* sp. NK10958. I. Taxonomy, production, isolation and preliminary Characterization. J. Antibiotics 47: 697~702, 1994
2) KOBAYASHI, S.; K. TSUCHIYA, T. KUROKAWA, T. NAKAGAWA, N. SHIMADA & Y. IITAKA: Pironetin, a novel plant growth regulator produced by *Streptomyces* sp. NK10958. II. Structural elucidation. J. Antibiotics 47: 703~707, 1994
3) KOBAYASHI, S.; K. TSUCHIYA, M. NISHIDE, T. NISHIKIORI, T. NAKAGAWA & N. SHIMADA: Pironetin, a novel plant growth regulator produced by *Streptomyces* sp. NK10958. III. Biosynthesis. J. Antibiotics 48: 893~895, 1995