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NEOVASIPYRIDONES A, B AND C: METABOLITES RELATED TO NEOVASININ, A PHYTOTOXIN OF THE FUNGUS, *NEOCOSMOSPORA VASINFECTA*

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Key Word Index—*Neocosmospora vasinfecta*; phytotoxins; phytopathogens; neovasinin; 2,3-dihydro-4-pyridinone.

Abstract—Three new metabolites, neovasipyridones A, B and C, were isolated from the phytopathogenic fungus *Neocosmospora vasinfecta* NHL2298, and their structures elucidated by spectroscopic methods. The biosynthetic relationship between the neovasipyridones and neovasinin, a phytotoxin of this fungus, is discussed.

INTRODUCTION

Neocosmospora vasinfecta E.F. Smith is a pathogen which causes root- and fruit-rot and seedling damping-off in the Malvaceae, Leguminosae, Piperaceae and Cucurbitaceae [1]. *Neocosmospora vasinfecta* NHL2298, isolated from the soil of Johannesburg (Republic of South Africa) produces neovasinin (**1**) which is phytotoxic to soybean, a host plant of this fungus [2, 3]. Neovasinin is a new type of fungal metabolite that has a unique bicyclic unit, 2*H*,5*H*-pyrano[4,3-*b*]pyran-2-one, and probably biosynthesized from a hexaketide and five C_1 units. To examine its biosynthetic pathway, particularly formation of the bicyclic unit, we undertook a search for metabolites biogenetically related to the neovasinin, produced by this fungus. Very recently we reported the structures of neovasipyrones A (**2a**) and B (**2b**) and those of neovasifuranones A (**3a**) and B (**3b**) [4]. We here report the structures of three new metabolites, named neovasipyridones A (**4a**), B (**4b**) and C (**4c**), which seem to be biogenetically related to neovasinin.

RESULTS AND DISCUSSION

Neovasipyridones A (**4a**), B (**4b**) and C (**4c**) were isolated as oils with respective yields of 0.036, 0.023 and 0.018 mg g^{-1} from air-dried mycelium of the fungus *N. vasinfecta* NHL 2298 grown on a malt extract medium supplemented with peptone and methionine [4, 5]. The ^{13}C NMR data (Table 1) and HRFAB mass spectrum of neovasipyridone A (**4a**) showed its molecular formula to be $C_{21}H_{35}NO_3$ (five unsaturations). The ^{13}C NMR spectrum of **4a** showed 21 resonances, seven of which were due to methyls, four to methylenes, five to methines and five to quaternary carbons from a DEPT experiment [6], indicating that one of the protons in the molecule is bound to oxygen or nitrogen. The 2,3-dihydro-4-pyridinone portion in **4a** was indicated by two IR absorption bands at 1647 and 1580 cm^{-1}, three ^{13}C resonances at δ191.7, 158.8 and 104.9 and a UV absorption band at 322 nm [7, 8]. Two ^{13}C resonances at δ127.6 and 140.1 were attributed to two olefinic carbons of a trisubstituted

Table 1. ^{13}C NMR data for **4a**, **4b** and **4c**

C	**4a**	**4b**	**4c**
2	76.7	76.1	76.3
3	71.1	70.9	71.0
4	191.7	191.6	191.7
5	104.9	104.8	104.8
6	158.8	158.7	158.1
7	127.6	127.7	127.5
8	140.1	140.0	140.1
9	34.0	34.0	34.0
10	30.1	30.1	30.1
11	11.8	11.8	11.8
12	13.1	13.0	13.1
13	20.6	20.6	20.6
14	28.8	28.6	28.9
15	197.6	197.6	197.6
16	34.9	34.8	34.9
17	8.5	8.6	8.5
18	61.2	53.6	62.7
19	33.7	37.6	27.2
20	26.4	25.7	19.8
21	10.8	22.0	20.1
22	16.8	22.5	—

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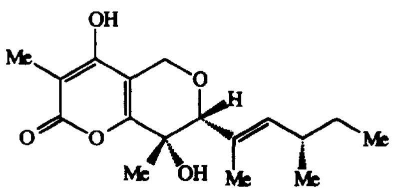

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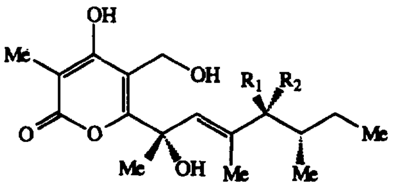
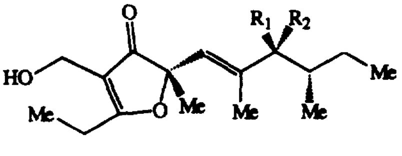
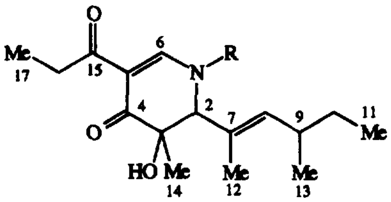


	R_1	R_2
2a	H	OH
2b	OH	H



	R_1	R_2
3a	H	OH
3b	OH	H



	R
4a	18 22 21 $-CH_2CH(Me)CH_2Me$
4b	18 21,22 $-CH_2CH_2CH(Me)_2$
4c	18 20,21 $-CH_2CH(Me)_2$

double bond. One ^{13}C resonance of δ197.6 was characteristic of a ketonic carbonyl carbon conjugated to a double bond. The structure of **4a** was unambiguously deduced from analysis of H,H-COSY [9, 10], C,H-COSY [11, 12], long-range C,H-COSY (COLOC) [11, 12] (Table 2) and NOE data (Table 3).

Table 2. COLOC data for **4a**

C	COLOC
3	H-2, H-14
4	H-6, H-2, H-14,
6	H-2
7	H-2, H-12
8	H-12
9	H-11, H-13
10	H-11, H-13
12	H-2, H-8
14	H-2
15	H-17
16	H-17
19	H-21
20	H-22

Table 3. NOE observed in **4a**

IRR	NOE
H-2	H-8, H-14, H-18b, H-19
H-6	H-18a*
H-8	H-2

*Refers to the methylene proton that resonates in the upper field.

The partial structure A (Fig. 1) was deduced from H,H-COSY and COLOC data. The structure of the side chain (C-7 to C-11), deduced to be 1,3-dimethyl-1-pentenyl from H,H-COSY, was confirmed by COLOC data. The bonds between C-2 and C-7, and between C-2 and C-3, were deduced from COLOC data. The allylic methyl carbon C-12 was correlated with the methine proton H-2, indicating the attachment of a 1,3-dimethyl-1-pentenyl group to the methine carbon, C-2. Furthermore, the oxygen-bearing quaternary carbon C-3, was correlated both with the methine proton H-2, and the methyl protons H-14, and the methyl carbon C-14 was in turn correlated with the methine proton H-2. These correlations indicated that the methyl carbon C-14 is bonded to the oxygen-bearing quaternary carbon C-3, which is attached to the methine carbon C-2. The stereochemistry of the double bond is *E* based on the NOE data, irradiation of the methine proton H-2 caused NOE enhancement of the signal of the olefinic proton H-8, and irradiation of the olefinic proton H-8 induced NOE enhancement of the signal of the methine proton H-2. The partial structure A therefore was established. The partial structure B (Fig. 1) also was deduced from H,H-COSY and COLOC data. An ethyl group (C-16 and C-17) was indicated by the ^1H NMR data and H,H-COSY. Attachment of the ethyl group to a ketonic carbonyl carbon C-15 was deduced from the COLOC correlation of the carbonyl carbon C-15 to the methyl protons H-17, thus giving the partial structure B. The partial structure C (Fig. 1) was shown to be 2-methylbutyl from the

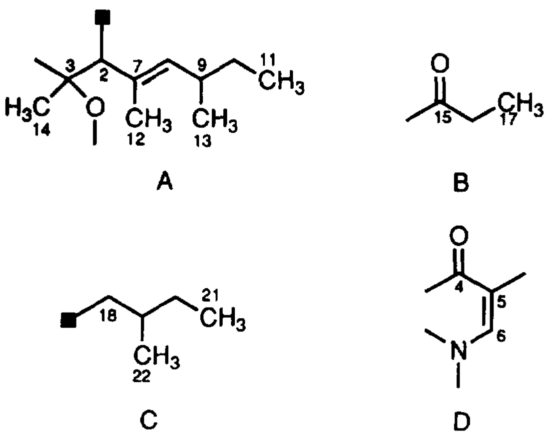


Fig. 1. Structural units of neovasipyridone A (**4a**).

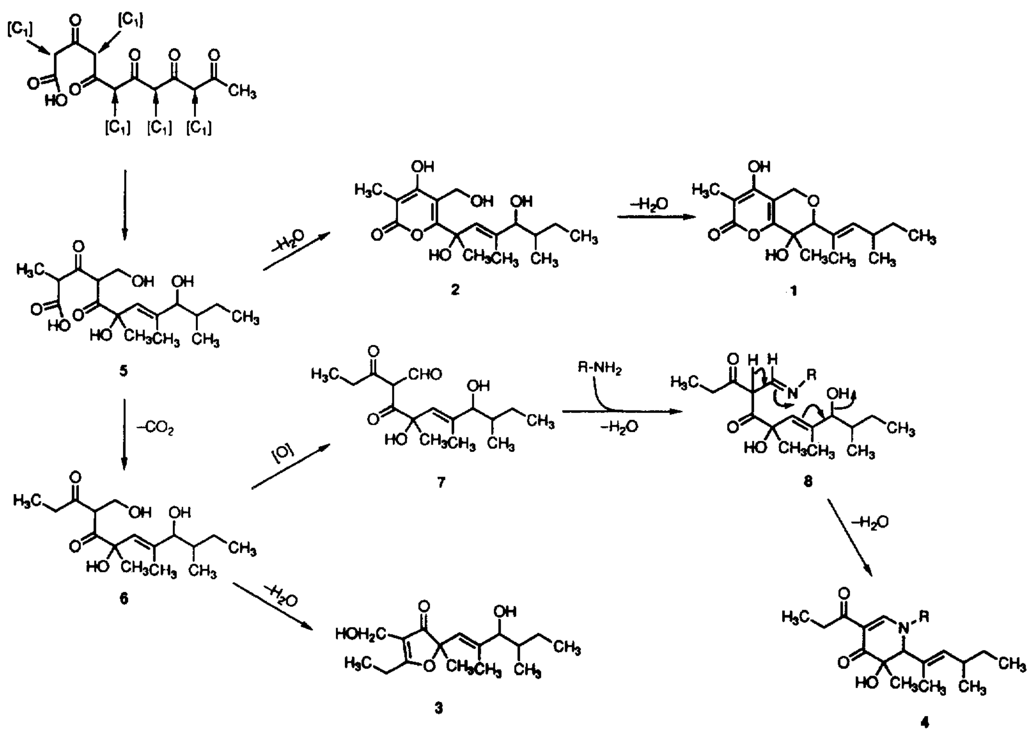
^{1}H NMR data and H,H-COSY. The partial structure D (Fig. 1) was deduced from the IR, UV and ^{13}C NMR data as described above.

These four partial structures A–D were combined on the basis of chemical shift considerations and the COLOC data. The carbonyl carbon C-4 in partial structure D had COLOC correlations with the methyl protons H-14 and with the methine proton H-2, both in partial structure A, indicating a bond between C-3 and C-4. The COLOC correlation of the olefinic carbon C-6 in partial structure D to the methine proton H-2 in partial structure A, and the chemical shift of the methine carbon C-2 (δ_C 76.7), were in agreement with there being a bond between C-2 and the nitrogen atom. The finished combination of partial structures A and D gave an integrated partial structure with a 2,3-dihydro-4-pyridinone ring. The chemical shift of the ketonic carbonyl carbon C-15 (δ_C 197.6) in partial structure B indicated bonding of the carbonyl carbon C-15 to the olefinic carbon C-5 on the 2,3-dihydro-4-pyridinone ring. The chemical shift of the methylene carbon (C-18, δ_C 61.2) and the methylene protons (H-18a, δ_H 3.10; H-18b, δ_H 3.23) indicated a bond between C-18 and the nitrogen atom. This was confirmed by the NOE observations. Irradiation of H-2 caused NOE enhancement of the signal of one of the methylene protons (H-18b), and irradiation of H-6 caused NOE enhancement of one of the methylene protons (H-18a). Finally, the one remaining hydrogen atom was bonded to the oxygen atom on C-3 to give the complete structure of **4a**. NOE enhancement of the signal of the methyl protons (H-14) on irradiation of the methine proton (H-2) suggested that the methyl group was pseudo-equatorial on the supposition that the H-2 proton was pseudo-axial. The stereochemistries of the other asymmetric carbons have yet to be resolved although the absolute stereochemistries of neovasinin [3] and the neovasipyrones [5] suggest that the stereochemistries of C-2, C-3 and C-9 in neovasipyridone A are (*R*,*S*,*S*).

Neovasipyridone B (**4b**) had the same molecular formula, $C_{21}H_{35}NO_3$ (HRFABMS and ^{13}C NMR data), as neovasipyridone A, and their UV spectra were very similar. Their 16 ^{13}C resonances due to C-2 to C-17 (Table 1) were nearly superimposable, indicating that neovasipyridone B has the same structure, except for the *N*-alkyl group, as neovasipyridone A. The ^{1}H NMR (Table 4) and H,H-COSY data indicated the attachment of 3-methylbutyl to the nitrogen atom on the 2,3-dihydro-4-pyridinone ring. Neovasipyridone C (**4c**) had a molecular formula of $C_{20}H_{33}NO_3$ (HREIMS and ^{13}C NMR data), which is equal to the loss of CH_2 from neovasipyridones A and B. The 16 ^{13}C resonances due to C-2 to C-17 (Table 1) were nearly superimposable on those of neovasipyridones A and B. The ^{1}H NMR (Table4) and H,H-COSY data indicated the 2-methylpropyl was a

Table 4. ^{1}H NMR data for **4a**, **4b** and **4c**

H	**4a**	**4b**	**4c**
2	3.82 *s*	3.80 *s*	3.80 *s*
6	8.21 *s*	8.25 *s*	8.20 *s*
8	5.12 *dq* (9.5, 0.9)	5.12 *dq* (10.8, 1.2)	5.13 *dq* (9.5, 1.0)
9	2.28 *m*	2.25 *m*	2.28 *m*
10	1.10–1.40 *m*	1.10–1.42 *m*	1.07–1.40 *m*
11	0.83 *t* (7.6)	0.82 *t* (7.5)	0.81 *t* (7.5)
12	1.43 *d* (0.9)	1.41 *d* (1.2)	1.41 *d* (1.0)
13	0.93 *d* (6.8)	0.91 *d* (6.8)	0.91 *d* (6.8)
14	1.38 *s*	1.34 *s*	1.37 *s*
16	2.86 *dq* (17.1, 7.2)	2.84 *dq* (16.5, 7.5)	2.84 *dq* (17.0, 7.0)
	2.98 *dq* (17.1, 7.2)	2.97 *dq* (16.5, 7.5)	2.97 *dq* (17.0, 7.0)
17	1.10 *t* (7.2)	1.09 *t* (7.5)	1.08 *t* (7.0)
18	3.10 *dd* (13.5, 8.6)	3.29 *dd* (13.5, 7.5)	2.94 *dd* (14.0, 9.0)
	3.23 *dd* (13.5, 5.8)	3.39 *dd* (13.5, 7.0)	3.28 *dd* (14.0, 5.2)
19	1.80 *m*	1.60 *m*	2.03 *m*
20	1.25 *m*	1.60 *m*	0.97 *d* (6.8)
21	0.95 *t* (7.2)	0.95 *d* (6.5)	0.98 *d* (6.8)
22	0.98 *d* (6.3)	0.96 *d* (6.5)	



Scheme 1. Proposed biosynthetic relationships between neovasinin (**1**) and the neovasipyridones (**4**), neovasipyrones (**2**) and neovasifuranones (**3**).

N-alkyl instead of the 2-methylbutyl in neovasipyridone A or the 3-methylbutyl in neovasipyridone B.

From their chemical structures, we propose the biosynthetic relationships between neovasinin (**1**), neovasipyridones (**4**), neovasipyrones (**2**) and neovasifuranones (**3**) shown in Scheme 1. The relationships between neovasinin, neovasipyrones and neovasifuranones has been discussed elsewhere [4]. Here, we discuss the relationship between neovasipyridones and neovasifuranones. Neovasipyridones and neovasifuranones are derived from a hexaketide chain plus five C_1 units via the hypothetical common intermediate **6**. Neovasipyridones (**4**) are formed from **6** through oxidation into aldehyde **7**, Schiff base formation with alkyl amines which seem to be from isoleucine, leucine and valine in the respective case of neovasipyridones A (**4a**), B (**4b**) and C (**4c**) then dehydration/cyclization. In contrast, neovasifuranones (**3**) are formed from **6** through dehydration/cyclization. A leaf spot bioassay indicated that the neovasipyridones like the neovasipyrones were not phytotoxic to soybean plants although neovasinin and neovasifuranones are phytotoxic.

EXPERIMENTAL

General. NMR spectra were recorded in $CDCl_3$ on a JEOL JNM GX-270 FT NMR spectrometer. NMR chemical shifts were referenced against $CDCl_3$ (δ_H 7.26, δ_C 77.00). MS were obtained with a JEOL DX-300 spectrometer. Glycerol was the matrix used for FABMS. In EIMS the ionization voltage was 70 eV. Fourier-transform IR spectra were obtained with a JASCO FT/IR-7000 spectrometer. Optical rotations were measured with a Horiba SEPA-200 high sensitive polarimeter. Daisogel IR-60 was the silica gel used for column chromatography. Prep. TLC was done on a Merck Kieselgel 60 HF_{254} glass plate (20 × 20 × 0.05 cm).

Fungus. The strain NHL2298 of *Neocosmospora vasinfecta* E.F. Smith var. *africana* (von Arx) Cannon et Hawksworth used in this study was a gift from Dr S. Udagawa of the National Institute of Hygienic Sciences (present address: Nodai Research Institute, Tokyo University of Agriculture, Setagaya-ku, Tokyo 156, Japan) in 1983 and has been maintained on potato dextrose agar.

Extraction and isolation of neovasipyridones A (**4a**), *B* (**4B**) *and C* (**4c**). The fungus was grown in a 500 ml conical flask containing liquid medium (200 ml × 200) composed of sucrose (50 g l^{-1}), peptone (3 g l^{-1}), the extract from 100 g l^{-1} on malt, L-methionine (300 mg l^{-1}) and H_2O, without shaking at 24° for 21 days in the dark. The mycelial mats obtained after filtering the culture broth were rinsed with H_2O and dried under a hood. The dried mats (620 g) were soaked in Me_2CO overnight, after which the Me_2CO extract was obtained by filtration. This treatment was repeated 3 ×. The combined Me_2CO extracts were evaporated to dryness. The residue was dissolved in 1 M $NaHCO_3$ (500 ml) and treated with EtOAc (500 ml). The EtOAc soln obtained was placed over Na_2SO_4 and evaporated to dryness. The residue was partitioned with $MeOH-H_2O$ (9:1, 250 ml) and *n*-hexane (250 ml). The *n*-hexane layer obtained was extracted with $MeOH-H_2O$ (9:1, 2 × 250 ml). The combined MeOH soln was evaporated to dryness. The residue (13.5 g) was subjected to silica gel column chromatography (340 g, 32 × 4.6 cm). The column was developed successively with 2000 ml (5 × 400 ml) each of 0%, 2%, 5%, 10% and 20% Me_2CO in *n*-hexane. Frs 1–3 eluted with 10% Me_2CO in *n*-hexane contained the neovasipyridones. The first fraction (455 mg) was purified by Sephadex LH-20 column (80 × 2.7 cm) chromatography with MeOH as the solvent. Each 7 ml of eluate constituted one fr., and frs 41–44 were combined and evaporated to dryness. The residue (30 mg) further was purified by prep. TLC [$Me_2CO-CHCl_3$ (5:95)] to afford neovasipyridone A (15.5 mg). The second and third fractions were combined and evaporated to dryness. The residue (1.29 g) was subjected to Sephadex LH-20 column chromatography (122 × 3.2 cm) with MeOH as the solvent. Each 10 ml of eluate constituted one fr., and frs 56–61 were combined and evaporated to dryness. The residue (73 mg) contained neovasipyridones A, B and C. Removal of neovasipyridone A from the mixture was achieved by prep. TLC [$EtOAc-C_6H_6$ (7:93), quadruple development], giving neovasipyridone A (6.7 mg) as a constituent of a higher R_f band on TLC. The lower R_f band contained neovasipyridones B and C. Neovasipyridone B was separated from C by repeated HPLC (column: DAISOPAK SP-120-5-ODS-A (250 × 10 mm), solvent: $MeOH-H_2O$ (9:1), flow rate: 1.0 ml min^{-1}, detector: 220 nm), giving neovasipyridones B (14.0 mg, R_t 28.0 min) and C (11.1 mg, R_t 33.0 min).

Neovasipyridone A (**4a**). Oil. $[\alpha]_D^{20}$ + 366° (EtOH; *c* 1.0). UV λ_{max}^{EtOH} nm (log ε): 202 (3.79), 262 (4.10), 322 (4.14). IR ν_{max}^{Film} cm^{-1}: 3398, 2964, 2930, 2874, 2858, 1647, 1580, 1462, 1375, 1350, 1315, 1267, 1241, 1172, 1114. ^1H and ^{13}C NMR spectral data: Tables 1 and 4. FABMS *m/z* (rel. int.): 350 $[M + H]^+$ (100), 332 (10), 306 (14), 236 (30), 196 (12); exact mass calcd for $C_{21}H_{36}NO_3$ 350.2695, found 350.2655.

Neovasipyridone B (**4b**). Oil. $[\alpha]_D^{20}$ + 392° (EtOH; *c* 1.0). UV λ_{max}^{EtOH} nm (log ε): 200 (3.71), 263 (4.15), 322 (4.19). IR ν_{max}^{Film} cm^{-1}: 3362, 2960, 2874, 1638, 1574, 1460, 1392, 1377, 1334, 1292, 1257, 1201, 1156, 1116, 1083, 1029. ^1H and ^{13}C NMR spectral data: Tables 1 and 4. FABMS *m/z* (rel. int.): 350 $[M + H]^+$ (100), 306 (15), 196 (10), 154 (39), 149 (27), 136 (33), 107 (15); exact mass calcd for $C_{21}H_{36}NO_3$ 350.2695, found 350.2666.

Neovasipyridone C (**4c**). Oil. $[\alpha]_D^{20}$ + 383° (EtOH; *c* 1.0). UV λ_{max}^{EtOH} nm (log ε): 203 (3.98), 262 (4.11), 323 (4.14). IR ν_{max}^{Film} cm^{-1}: 3406, 2967, 2926, 2874, 1639, 1579, 1460, 1369, 1315, 1261, 1157, 1113. ^1H and ^{13}C NMR spectral data: Tables 1 and 4. EIMS *m/z* (rel. int.): 335 $[M]^+$ (55), 292 (100), 278 (23), 222 (15), 182 (41), 125 (19); exact mass calcd for $C_{20}H_{33}NO_3$ 335.2461, found 335.2488.

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