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NEOVASIPYRONES AND NEOVASIFURANONES: FOUR NEW METABOLITES RELATED TO NEOVASININ, A PHYTOTOXIN OF THE FUNGUS, *NEOCOSMOSPORA VASINFECTA*

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Key Word Index—*Neocosmospora vasinfecta*; phytotoxins; phytopathogens; neovasinin; 2-pyrones; 3-furanones.

Abstract—Four new metabolites, neovasipyrones A and B and neovasifuranones A and B, were isolated from the phytopathogenic fungus *Neocosmospora vasinfecta* NHL2298, and their structures were elucidated by spectroscopic methods. The absolute stereochemistry of the neovasipyrones was established by X-ray analysis and chemical reactions.

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 was isolated from the soil of Johannesburg in South Africa and found to produce neovasinin (**1**), which is phytotoxic against soybean, one of the host plants of this fungus [2, 3].

Compound **1** is a new type of fungal metabolite containing a unique bicyclic unit, 2*H*,5*H*-pyrano[4,3-*b*]pyran-2-one, and seems likely to be biosynthesized from a hexaketide and five C_1 units. To explore its biosynthetic pathway, particularly the formation of the bicyclic unit, we undertook a search for the biogenetically related metabolites to **1** produced by this fungus. In this paper, we report the structures of four new metabolites, named neovasipyrones A (**2a**) and B (**2b**) and neovasifuranones A (**4a**) and B (**4b**), and discuss the possible biosynthetic relationships between them and **1**.

RESULTS AND DISCUSSION

Neocosmospora vasinfecta NHL 2298 produced **2a** and **2b** in trace amounts on the conventional malt–peptone–sucrose medium which had been used for production of **1**. Addition of methionine to this medium, according to our previous report on the biosynthesis of secondary metabolites in another fungus [4], stimulated a dramatic production of **2a** and **2b** as well as **1**. In this study we adopted this improved culture condition.

Compounds **2a** and **2b** in the acidic ethyl acetate extract of *N. vasinfecta* culture filtrate were purified by silica gel partition, silica gel and Sephadex LH-20 column chromatography, successively. The separation of **2a** from **2b** was achieved by preparative TLC to afford **2a** and **2b** both as pure compounds in yields of 72 and 31 mg l^{-1}, respectively. Compounds **4a** and **4b** in the neutral ethyl acetate extract were purified by silica gel and Sephadex LH-20 column chromatography, successively. The separation of **4a** from **4b** was also achieved by preparative TLC to afford **4a** and **4b** both in a pure state in yields of 3.0 and 1.9 mg l^{-1}, respectively.

Compound **2a** had a molecular formula of $C_{17}H_{26}O_6$ (HRFABMS and ^{13}C NMR data). The ^{13}C NMR spectrum of **2a** (Table 1) showed 17 resonances, five of which were due to methyls, two to methylenes, three to methines and seven to quaternary carbons from a DEPT experiment [5], indicating four hydroxyls in **2a**. The five ^{13}C resonances (δ_c 100.6, 110.4, 163.5, 164.9, 167.7), together with the UV absorption at 291 nm and the IR absorption bands (3373, 1663 and 1567 cm^{-1}), indicated a trisubstituted 4-hydroxy-2*H*-pyran-2-one moiety in **2a** [2, 6, 7]. Two ^{13}C resonances (δ_c 132.7 and 140.3) were attributed to two olefinic carbons in a trisubstituted double bond. The structure of **2a** was unambiguously deduced from analysis of H,H-COSY [8, 9], C,H-COSY [10, 11], long-range C,H-COSY (COLOC) [10, 11] and NOE data. The COLOC spectrum of **2a** was especially helpful in the assignment of the structure. The significant correlations in the COLOC spectrum are summarized in Fig. 1.

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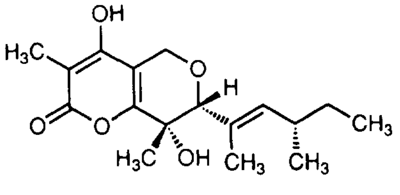

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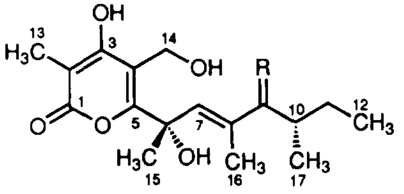
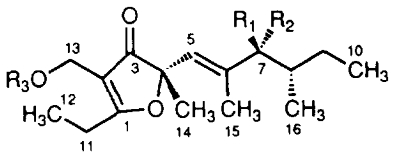


2a R= α-OH, β-H
2b R= α-H, β-OH
3 R= O



4a R_1= H, R_2= OH, R_3= H
4b R_1= OH, R_2= H, R_3= H
5 R_1=H, R_2=OAc, R_3= Ac

Table 1. NMR data (^1H, 270.05 MHz and ^{13}C, 67.80 MHz) for neovasipyrones A (**2a**) and B (**2b**) in acetone-d_6

Carbon no.	**2a** δ_C	**2a** δ_H	**2b** δ_C	**2b** δ_H
1	164.9		164.7	
2	100.6		100.7	
3	167.7		167.5	
4	110.4		110.7	
5	163.5		163.3	
6	75.5		75.2	
7	132.7	5.74 *dq* (1.1, 1.2)	134.7	5.69 *dq* (0.7, 1.3)
8	140.3		140.0	
9	80.8	3.66 *dd* (1.1, 5.8)	83.2	3.54 *br.d* (8.6)
10	38.9	1.46 *m*	38.9	1.40 *m*
11	27.6	1.05 *m* 1.35 *m*	26.1	1.08 *m* 1.71 *m*
12	12.4	0.83 *t* (7.7)	11.9*	0.82 *t* (7.4)
13	9.1	1.81 *s*	9.1	1.82 *s*
14	58.8	4.80 *d* (13.1) 4.88 *d* (13.1)	58.7	4.85 *d* (13.4) 4.88 *d* (13.4)
15	28.7	1.54 *s*	28.1	1.55 *s*
16	13.8	1.43 *d* (1.2)	12.4*	1.39 *d* (1.3)
17	14.7	0.81 *d* (6.9)	16.4	0.70 *d* (6.7)

^1H NMR data represent chemical shift, multiplicity (*J* in Hz).
*Assignments may be interchanged.

The structure of the side chain (C-6 to C-12) was deduced from H,H-COSY and COLOC data. The H,H-COSY data allowed the construction of a 1-hydroxy-2-methylbutyl group. Additionally, the olefinic proton, 7-H (δ_H 5.74) had the allylic correlations with both the hydroxymethine proton, 9-H (δ_H 3.66) and the methyl protons, 16-H_3 (δ_H 1.43), indicating the attachment of a 1-hydroxy-2-methylbutyl group and a methyl group to the olefinic carbon, C-8, in the double bond. The stereochemistry around the double bond was assigned as *E* on the basis of NOE data. Irradiation of the signal of the olefinic proton, 7-H (δ_H 5.74), caused a NOE enhancement on the signal of the hydroxymethine, 9-H (δ_H 3.66), but had no effect on the signal of the methyl protons, 16-H_3 (δ_H 1.43). The connection of C-6 and C-7 was deduced from COLOC data as follows. First, the attachment of the methyl group, C-15, to the hydroxy quaternary carbon, C-6, was quite obvious from ^1H and ^{13}C NMR data. The two carbons, C-6 and C-7, had correlations with the methyl protons, 15-H_3, and in addition, C-6 had a correlation with the olefinic proton, 7-H. Thus, the establishment of the structure of the side chain was completed.

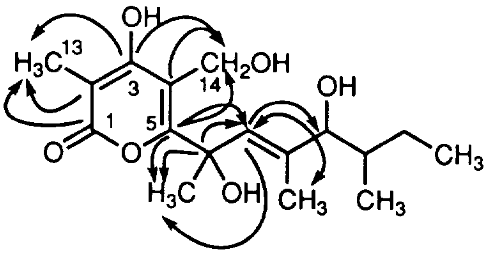


Fig. 1. Key long-range couplings detected in the COLOC experiment of neovasipyrone A (**2a**).

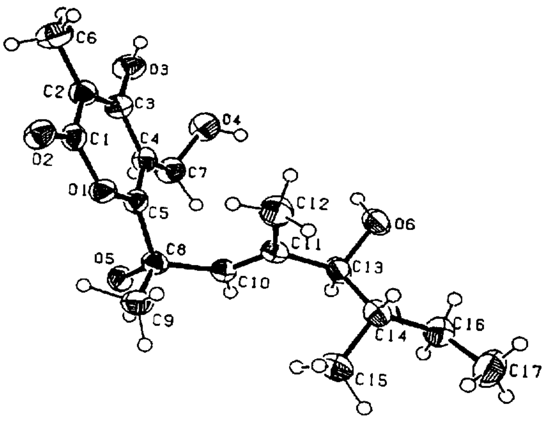


Fig. 2. Perspective view of neovasipyrone B (**2b**) drawn by ORTEP-II [17] and the numbering system used for X-ray molecular structure.

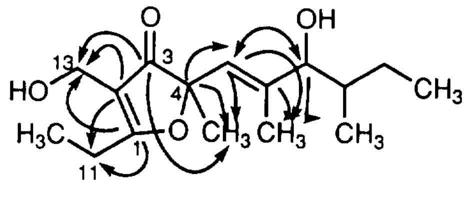


Fig. 3. Key long-range couplings detected in the COLOC experiment of neovasifuranone A (**4a**).

The structure of the 4-hydroxy-2*H*-pyran-2-one portion was then established through the analysis of COLOC data. Three carbons, C-1, C-2 and C-3, were correlated with the methyl protons, 13-H_3, and also three carbons, C-3, C-4 and C-5, were correlated with the hydroxymethyl protons, 14-H_2. C-5 had correlations with both the olefinic proton, H-7, and the methyl protons, 15-H_3. These correlations allowed the complete assignment of the 4-hydroxy-2*H*-pyran-2-one portion and, consequently, the entire structure was established for **2a**.

Compound **2b** had a molecular formula of $C_{17}H_{26}O_6$ (HRFABMS and ^{13}C NMR data) and the same structure as **2a** from their NMR data comparison (Table 1), i.e. they were diastereomer of each other. The oxidation of **2a** with Jones reagent gave a 9-oxo derivative (**3**), which was identical with the derivative from **2b** in all respects. This indicated that **2a** and **2b** were different only in the stereochemistry at C-9. To establish their stereochemistry by X-ray analysis, crystallization of **2a** was attempted, but was unsuccessful. Fortunately, **2b** was crystallized from acetone–benzene to afford plates containing one equivalent of benzene, and the crystals were adequate for X-ray analysis. The molecular structure of **2b** is shown in Fig. 2. The relative configuration of **2b** was found to be (6*S**, 9*S**, 10*S**) and thus that of **2a** was (6*S**, 9*R**, 10*S**). Degradation of **2a** was carried out to establish its absolute stereochemistry. The reaction of **2a** with OsO_4 and $NaIO_4$, followed by treatment with 2,4-dinitrophenylhydrazine, afforded the 2,4-dinitrophenylhydrazone of 2-methyl-1-butanal. Its optical rotation (+24.7°) was identical with that of the synthetic 2,4-dinitrophenylhydrazone of (2*S*)-2-methyl-1-butanal (+23.3°) [3], indicating that the configuration of C-10 in **2a** was *S*. Consequently, the absolute stereochemistries of **2a** and **2b** are (6*S*, 9*R*, 10*S*) and (6*S*, 9*S*, 10*S*), respectively.

Compound **4a** had a molecular formula of $C_{16}H_{26}O_4$ (HREIMS and ^{13}C NMR data). The ^{13}C NMR spectrum of **4a** (Table 2) showed 16 resonances, five of which were due to methyls, three to methylenes, three to methines and five to quaternary carbons, indicating two hydroxyls in **4a**. The four ^{13}C resonances (δ_c 91.0, 114.1, 194.0 and 208.2), together with the UV absorption at 268 nm and the IR absorption bands (1688 and 1611 cm^{-1}), indicated a tetrasubstituted 2,3-dihydrofuran-3-one portion in **4a** [12–14]. Two ^{13}C resonances (δ_c 124.4 and 146.0) were attributed to two olefinic carbons in a trisubstituted double bond. The structure of **4a** was unambiguously deduced from analysis of H,H-COSY, C,H-COSY, COLOC and NOE data in the same manner as in the structural elucidation of **2a**. The significant correlations in the COLOC spectrum are summarized in Fig. 3. Acetylation of **4a** afforded a diacetyl derivative (**5**), in the ^1H NMR spectrum of which two proton resonances associated with a hydroxy methine and a hydroxy methyl were shifted downfield (from δ3.68 to 4.92 and from δ4.20 to 4.64 and 4.66), indicating that the two carbons, C-7 and C-13, in **4a** bear free hydroxy groups.

The structure of the side chain (C-5 to C-10) was deduced to be 3-hydroxy-2,4-dimethyl-1-hexenyl from H,H-COSY and COLOC data. The stereochemistry of the double bond was assigned as *E* on the basis of NOE data. Irradiation of the signal of the olefinic proton, 5-H (δ_H 5.45) caused a NOE enhancement on the signal of the hydroxymethine, 7-H (δ_H 3.68), but had no effect on the signal of the methyl protons, 15-H_3 (δ_H 1.70). A methyl, an ethyl and a hydroxymethyl group in **4a** were deduced from ^1H NMR data. The substitution pattern around the 2,3-dihydrofuran-3-one moiety was established on the basis of COLOC data. The three carbons, C-1, C-2 and C-3, were correlated with the hydroxymethyl protons, 13-H_2, and the two carbons, C-1 and C-2 were correlated with the methylene protons, 11-H_2. The three carbons, C-3, C-4 and C-5, had correlations with the methyl protons, 14-H_3, and furthermore, the carbon C-4 had a correlation with the olefinic proton, 5-H. These correlations

Table 2. NMR data (^1H, 270.05 MHz and ^{13}C, 67.8 MHz) for neovasifuranones A (**4a**) in CD_3OD and B (**4b**) in $CDCl_3$

Carbon no.	**4a** δ_C	**4a** δ_H	**4b** δ_C	**4b** δ_H
1	194.0		189.5	
2	114.1		111.9	
3	208.2		205.6	
4	91.0		88.5	
5	124.4	5.45 *dq* (1.0, 1.1)	123.7	5.44 *dq* (1.2, 1.0)
6	146.0		143.5	
7	82.3	3.68 *dd* (1.0, 6.5)	82.1	3.66 *br.d* (7.7)
8	39.5	1.49 *m*	37.5	1.50 *m*
9	28.1	1.08 *m* 1.38 *m*	24.4*	1.10 *m* 1.66 *m*
10	12.7	0.90 *t* (7.3)	11.1†	0.89 *t* (7.3)
11	24.2	2.69 *dq* (14.6, 7.6) 2.75 *dq* (14.6, 7.6)	22.5*	2.62 *dq* (13.9, 7.5) 2.67 *dq* (13.9, 7.5)
12	11.6	1.25 *t* (7.6)	10.6†	1.25 *t* (7.5)
13	53.2	4.20 *s*	53.4	4.31 *s*
14	25.2	1.47 *s*	23.9	1.51 *s*
15	14.8	1.70 *d* (1.1)	12.8†	1.73 *d* (1.0)
16	15.2	0.84 *d* (6.7)	15.6	0.73 *d* (6.8)

^1H NMR data represent chemical shift, multiplicity (*J* in Hz).
*,†Assignments may be interchanged.

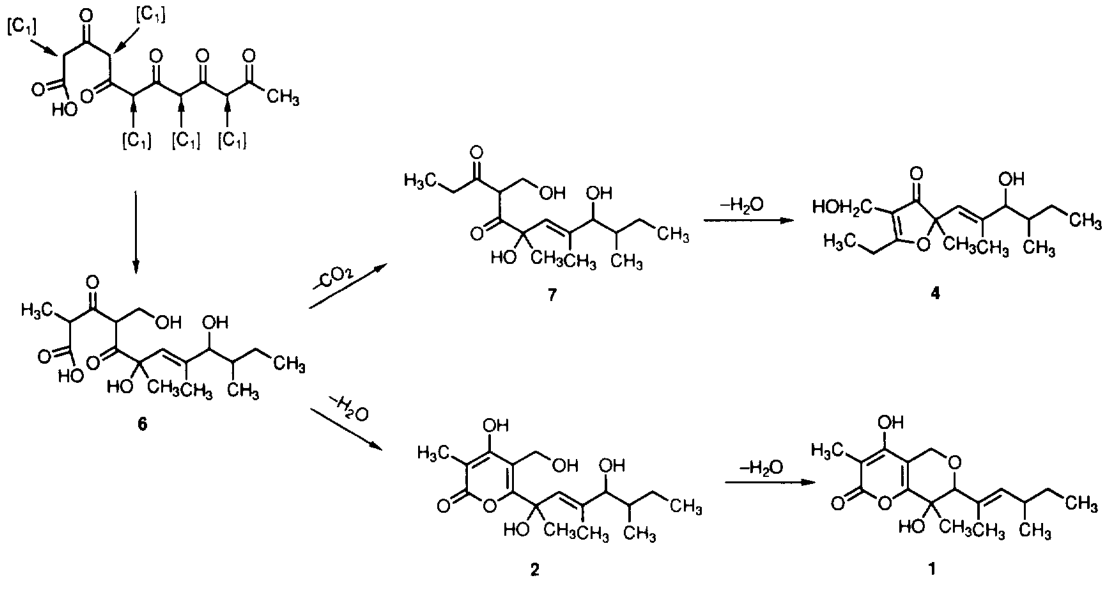


Fig. 4. Proposed biosynthetic pathway of neovasinin **1** and biosynthetic relationship between neovasipyrones **2** and neovasifuranones **4**.

allowed the complete assignment of **4a** and the entire structure was established.

From a comparison of NMR data (Table 2), **4b** had the same structure as **4a**. The stereochemistry of the neovasifuranones was most likely to be as shown in the figure from the comparison of the coupling constants, $J_{7,8}$ of **4a** and **4b** with $J_{9,10}$ of **2a** and **2b**, and also from the biogenetic point of view as discussed later. It is noteworthy that this fungus produces two types of biogenetically related diastereomers simultaneously, perhaps by the enzyme-catalysed reactions.

Following examination of the chemical structures reported herein, we propose a biosynthetic pathway of **1** and a biosynthetic relationship between neovasipyrones and neovasifuranones, as shown in Fig. 4.

Neovasipyrones and neovasifuranones are derived from a hexaketide chain plus five C_1 units via a hypothetical common intermediate **6**. Neovasipyrones (**2**) are formed from **6** through dehydration/cyclization and then subjected to further dehydration/cyclization reaction to yield **1**. On the other hand, neovasifuranones (**4**) are formed from **6** via the intermediate **7** through decarboxylation and dehydration/cyclization. Preliminary leaf spot bioassay indicated that **4** were phytotoxic to soybean, but **2** were not. Compound **1** caused chlorosis at 1 μg per plant and chlorosis followed by necrosis at 2 μg per plant; **4a** produced no symptom at 2 μg per plant, but caused necrosis at 5 μg per plant.

EXPERIMENTAL

General. NMR spectra were recorded on a JEOL JNM GX-270 FT NMR spectrometer or a JEOL JNM GX-400 FT NMR spectrometer. All NMR chemical shifts were referenced against the deuterated solvent used ($CDCl_3$, δ_H 7.26, δ_c 77.00; Me_2CO-d_6, δ_H 2.00, δ_c 30.30; CD_3OD, δ_H 3.30, δ_c 49.80). MS were obtained on a JEOL DX-300 spectrometer. In FABMS, glycerol was used as matrix. FT-IR spectra were obtained on a JASCO FT/IR-7000 spectrometer. Optical rotations were measured on a Horiba SEPA-200 high sensitive polarimeter. Unless otherwise mentioned, Daisogel IR-60 was used as the silica gel for CC. Prep. TLC was carried out on a Merck Kieselgel 60 HF_{254} glass plate (20 × 20 × 0.05 cm).

Fungus. 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 (National Institute of Hygienic Sciences) in 1983 and was maintained on potato dextrose agar.

Extraction of metabolites. The fungus was grown in a 500 ml conical flask containing a liquid medium (200 ml × 50) made up of sucrose (50 $g l^{-1}$), peptone (3 $g l^{-1}$), the extract from 100 $g l^{-1}$ of malt, L-methionine (300 $mg l^{-1}$) and H_2O without shaking at 24° for 21 days in the dark. The culture filtrate was acidified to pH 2.0 with HCl and extracted with EtOAc (3 × 7 l). The EtOAc extracts were dried over Na_2SO_4, concd, washed with 1 M $NaHCO_3$ (2 × 0.5 vol.) to afford the neutral EtOAc-soluble (NE) fraction. The $NaHCO_3$ washings were acidified to pH 2.0 with HCl and extracted with EtOAc (3 × 1 vol.) to afford the acidic EtOAc-soluble (AE) fraction.

Isolation of neovasipyrones A (**2a**) *and B* (**2b**). The residue (5.7 g) from AE fraction was purified by silica gel partition CC (300 g, impregnated with 180 ml of 0.1 M HCO_2H, 44 × 4.6 cm). Elution was performed with each 1.5 l (5 × 300 ml) of 10, 20, 30, 40 and 50% EtOAc in *n*-hexane satd with 0.1 M HCO_2H. Frs 15–21 were combined and concd. The residue (1.9 g) was subjected to silica gel CC (180 g, 44 × 3.2 cm). The column was developed with Me_2CO–C_6H_6–HOAc (40:60:0.5) and each 100 ml of eluate was collected as one fr. The combined frs (5–10, 1.6 g) were then subjected to Sephadex LH-20 CC (135 × 3.2 cm) using $CHCl_3$–MeOH (1:1) as solvent. Each 10 ml of eluate was collected as one fr., and frs 60–80, which contained both **2a** and **2b**, were combined. The sepn of **2a** from **2b** was achieved by prep. TLC [Me_2CO–$CHCl_3$–HOAc (25:75:1), quadruple development] to afford 720 mg of **2a** and 310 mg of **2b**.

Isolation of neovasifuranones A (**4a**) *and B* (**4b**). The combined NE fractions (5.9 g) from 5 cultivations (5 × 10 l of medium) were subjected to silica gel CC (140 g, 26 × 3.6 cm). The column was developed successively with 900 ml each of 2, 5, 10, 20, 30, 40, 50% Me_2CO in *n*-hexane. The fr. eluted with 30% Me_2CO in *n*-hexane contained neovasifuranones and was purified by Sephadex LH-20 CC (123 × 3.2 cm) using MeOH as solvent. Each 10 ml of eluate was collected as one fr., and frs 60–69 were combined and concd. The residue (850 mg) was further purified by prep. TLC [Me_2CO–$CHCl_3$(1:3), × 3] to afford **4a** (152 mg) and **4b** (97 mg).

Neovasipyrone A (**2a**). Oil. $[\alpha]_D^{20}$ $-235.4°$ (EtOH; *c* 1.0). UV λ_{max}^{EtOH} nm (log ε): 209 (4.29), 291 (3.90). IR ν_{max}^{film} cm^{-1}: 3373, 2963, 1663, 1567, 1414, 1244, 988. ^1H and ^{13}C NMR spectral data: Table 1. FABMS *m/z* (rel. int): 327 $[M + H]^+$ (39), 291 (35), 249 (26), 205 (36), 191 (40), 179 (33), 109 (58), 43 (100); exact mass calcd for $C_{17}H_{27}O_6$ 327.1808, found 327.1791.

Neovasipyrone B (**2b**). Oil. $[\alpha]_D^{20}$ $-124.0°$ (EtOH; *c* 1.0). UV λ_{max}^{EtOH} nm (log ε): 209 (4.25), 2.91 (3.87). IR ν_{max}^{film} cm^{-1}: 3373, 2967, 1680, 1565, 1419, 1245, 1010. ^1H and ^{13}C NMR spectral data: Table 1. FABMS *m/z* (rel. int): 327 $[M + H]^+$ (30), 291 (12), 249 (16), 205 (15), 179 (12), 109 (18), 106 (26), 74 (44), 42 (100); exact mass calcd for $C_{17}H_{27}O_6$ 327.1808, found 327.1804.

Crystal data for neovasipyrone B (**2b**). $C_{17}H_{26}O_6 \cdot C_6H_6$, $M = 404.5$, orthorhombic, space group $P2_12_12_1$, $a = 24.773(4)$, $b = 11.525(2)$, $c = 7.961(1)$ Å, $V = 2272.9(4)$ Å^3 (cell parameters by least squares from the setting angles of 24 reflections), $\mu(Cu\ K_\alpha) = 6.5\ cm^{-1}$, $Z = 4$, $D_x = 1.18\ g\,cm^{-3}$, $F(000) = 872$, $T = 293$ K.

X-ray crystal structure analysis. Intensity data were measured on a Rigaku four-circle diffractometer using Ni-filtered Cu K_α radiation ($\lambda = 1.5418$ Å) and a rotating anode generator. A crystal of dimensions 0.6 × 0.4 × 0.2 mm was used. The ω–2θ scan mode was employed, with background measurement at each end of the scan. Intensities of 1952 unique reflections were measured to $2\theta_{max} = 120°$ in the range $0 \leq h \leq 27$, $0 \leq k \leq 12$, $0 \leq l \leq 8$, with ω scan width of $0.9° + 0.15° \tan\theta$, a scan speed of 4° min^{-1}, and background counting time of 5 sec. No significant change was observed in the intensities of the 3 standard reflections measured every 100 reflections. Intensity data were corrected only for Lorentz and polarization effects. The structure was determined by direct methods using SHELX 86 [15] and refined by block diagonal least squares [16] on *F* using atomic scattering factors from International Tables for X-Ray Crystallography (1974). All H atoms for **2b** molecule were located in the difference Fourier map, but those for the C_6H_6 molecule were invisible in the map. The oxygen and carbon atoms were refined anisotropically and the H atoms isotropically. Strongest reflection 0 2 0, was omitted from the refinement. Weighting scheme used in the final stage of refinement was $w = [\sigma(F_0)^2 +$

$0.02F_0 + 0.001F_0^2]^{-1}$. The residual electron densities in the final difference Fourier map ranged from -0.19 to $0.19\,e\text{Å}^{-3}$. The final R and R_w were 0.061 and 0.080, respectively, for 1728 unique reflections with $F_0 > 2\sigma(F_0)$. Atomic coordinates, temp. factors and bond lengths and angles have been deposited at the Cambridge Crystallographic Data Centre.

Oxidation of neovasipyrones **2a** *and* **2b**. A soln composed of CrO_3 (500 mg), conc. H_2SO_4 (500 µl) and H_2O (1.5 ml) was prepared and 50 µl were added to a stirred soln of **2a** (36.0 mg) in 1.5 ml of Me_2CO at 0°. After 2 min, 20 µl of *iso*-PrOH was added to the reaction mixt. After 1 min, the reaction mixt. was diluted with 30 ml of H_2O and the soln was extracted with EtOAc (3 × 30 ml). The EtOAc soln was washed with brine, dried over Na_2SO_4, filtered and concd under red. pres. The residue was subjected to prep. TLC [Me_2CO–$CHCl_3$–HOAc (20:80:1)] to afford 6.4 mg of compound **3** as an oil. $[\alpha]_D^{22}$ $-96°$ (EtOH; *c* 1.0). ^{1}H NMR (270 MHz, $CDCl_3$): δ0.85 (3H, *t*, $J = 7.0$ Hz, H-12), 1.05 (3H, *d*, $J = 7.0$ Hz, H-17), 1.38 (1H, *m*, H-11), 1.59 (3H, *s*, H-15), 1.63 (1H, *m*, H-11), 1.76 (3H, *br s*, H-16), 1.87 (3H, *s*, H-13), 3.11 (1H, *ddq*, $J = 7.0$, 7.0, 7.0 Hz, H-10), 4.63 (1H, *d*, $J = 13.5$ Hz, H-14), 4.95 (1H, *d*, $J = 13.5$ Hz, H-14), 6.73 (1H, *br. s*, H-7). EIMS *m/z* (rel. int): 324 $[M]^+$ (3), 222 (8), 194 (42), 182 (51), 140 (100), 57 (33). FABMS *m/z* (rel. int): 325 $[M + H]^+$ (100), 307 (15), 291 (11), 249 (13), 223 (19), 183 (25), 57 (37). Compound **2b** (31.9 mg) was subjected to the same procedure as for **2a** to afford compound **3** (5.4 mg). $[\alpha]_D^{22}$ $-83°$ (EtOH; *c* 1.0).

2,4-*Dinitrophenylhydrazone of* 2-*methyl*-1-*butanal from* **2a**. A soln of **2a** (118 mg), OsO_4 (5 mg) and $NaIO_4$ (221 mg) in dioxane (2 ml), H_2O (1.5 ml) and pyridine (0.1 ml) was stirred for 12 hr at room temp. The reaction mixture was diluted with 20 ml of EtOAc, and the EtOAc soln was washed with brine (4 × 10 ml). The soln of 2,4-dinitrophenylhydrazine (308 mg) in EtOH (1.5 ml) and conc. H_2SO_4 (1 ml) was added to the EtOAc soln at 0°, and the reaction mixt. was stirred for 20 min. The reaction mixt. was washed with brine (2 × 10 ml), dried over Na_2SO_4 and concd to dryness. The residue was purified with silica gel flash CC [Wakogel FC-40, EtOAc–*n*-hexane (1:9)] and prep. HPLC (Whatman Partisil-5 ODS-2, 85% aq. MeOH) to afford 5.5 mg of 2,4-dinitrophenylhydrazone of 2-methyl-1-butanal as yellow solid. The spectroscopic data were in accordance with those reported previously. $[\alpha]_D^{22}$ $+24.7°$ ($CHCl_3$; *c* 0.3).

Neovasifuranone A (**4a**). Oil. $[\alpha]_D^{22}$ $-111.2°$ (EtOH; *c* 0.4). UV λ_{max}^{EtOH} nm (log ε): 202 (3.91), 268 (3.98). IR ν_{max}^{film} cm^{-1}: 3405, 2965, 1688, 1611, 1445, 1408, 1277, 1096, 1053, 1017. ^{1}H and ^{13}C NMR spectral data: Table 2. EIMS *m/z* (rel. int): 282 $[M]^+$ (5), 264 (13), 246 (8), 235 (7), 225 (19), 207 (79), 189 (17), 151 (49), 121 (28), 111 (62), 109 (100), 57 (63); exact mass calcd for $C_{16}H_{26}O_4$ 282.1831, found 282.1837.

Neovasifuranone B (**4b**). Oil. $[\alpha]_D^{22}$ $-102.3°$ (EtOH; *c* 0.4). UV λ_{max}^{EtOH} nm (log ε): 202 (3.93), 268 (4.01). IR ν_{max}^{film} cm^{-1}: 3356, 2965, 1688, 1611, 1464, 1412, 1277, 1115, 1051, 1011. ^{1}H and ^{13}C NMR spectral data: Table 2. EIMS *m/z* (rel. int): 282 $[M]^+$ (9), 264 (12), 246 (5), 235 (3), 225 (14), 207 (90), 189 (15), 151 (48), 121 (21), 111 (68), 109 (100), 57 (62): exact mass calcd for $C_{16}H_{26}O_4$ 282.1831, found 282.1831.

Diacetyl neovasifuranone A (**5**). A soln of **4a** (4.8 mg) in pyridine (0.1 ml) and Ac_2O (0.05 ml) was allowed to stand for 12 hr at room temp. The reaction mixt. was added to H_2O and the soln was stirred for 5 hr. The products were extracted with EtOAc (3 × 3 ml). The EtOAc soln was dried over Na_2SO_4 and concd to dryness under red. pres. The residue was purified by prep. TLC [Me_2CO–C_6H_6 (1:4), ×2] to afford **5** (5.1 mg) as an oil. UV λ_{max}^{EtOH} nm (log ε): 202 (4.02), 266 (4.13). IR ν_{max}^{film} cm^{-1}: 2969, 1742, 1709, 1626, 1464, 1412, 1373, 1236, 1022. ^{1}H NMR (400 MHz, $CDCl_3$); δ0.73 (3H, *d*, $J = 6.6$ Hz, H-16), 0.82 (3H, *t*, $J = 7.2$ Hz, H-10), 1.04 (1H, *m*, H-9), 1.16 (3H, *t*, $J = 7.5$ Hz, H-12), 1.25 (1H, *m*, H-9), 1.41 (3H, *s*, H-14), 1.57 (1H, *m*, H-8), 1.67 (3H, *d*, $J = 1.3$ Hz, H-15), 1.96 (3H, *s*, Me of Ac), 2.00 (3H, *s*, Me of Ac), 2.59 (1H, *dq*, $J = 14.7$, 7.5 Hz, H-11), 2.62 (1H, *dq*, $J = 14.7$, 7.5 Hz, H-11), 2.62 (1H, *dq*, $J = 14.7$, 7.5 Hz, H-11), 4.64 (1H, *d*, $J = 12.2$ Hz, H-13), 4.66 (1H, *d*, $J = 12.2$ Hz, H-13), 4.92 (1H, *br d*, $J = 6.3$ Hz, H-7), 5.35 (1H, *dq*, $J = 1.3$, 1.3 Hz, H-5). EIMS *m/z* (rel. int): 366 $[M]^+$ (3), 306 (18), 249 (100), 217 (57), 207 (67), 179 (34), 151 (19), 138 (38), 109 (28).

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