STUDIES ON NEW VASODILATORS, WS-1228 A AND B

II. STRUCTURE AND SYNTHESIS

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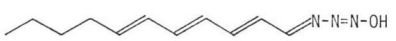
(Received for publication October 26, 1981)

The structure of new hypotensive vasodilators, WS-1228 A and B, produced by *Streptomyces aureofaciens*, were determined as **1** and **2**, respectively, on the basis of their spectral and chemical evidences.

WS-1228 A (**1**), having *N*-hydroxytriazene moiety, was synthesized from (E,E,E)-2,4,7-undecatrienal (**4**) by condensation with hydrazine hydrate followed by nitrosation.

In the course of screening for new biologically active compounds, we found that *Streptomyces aureofaciens* produces hypotensive vasodilators designated as WS-1228 A (**1**) and B (**2**). Taxonomy, isolation and characterization of these compounds have been reported in the preceding paper[1]. This report describes the structure elucidations of WS-1228 A (**1**) and B (**2**) and the synthesis of **1**.

WS-1228 A (1)



WS-1228 B (2)

WS-1228 A (**1**) was isolated as yellow needles [mp 100~102°C (dec.)] which showed a positive color reaction to ferric chloride reagent. Elemental analysis and mass spectrum established the molecular formula of **1** as $C_{11}H_{17}N_3O$. Absorption bands at 1612 and 1580 cm^{-1} in its IR spectrum (Fig. 1) and at 300 nm in its UV spectrum suggested the presence of the triene oxime moiety. The PMR spectrum (Fig.

Fig. 1. IR spectrum of WS-1228 A (**1**) (Nujol).

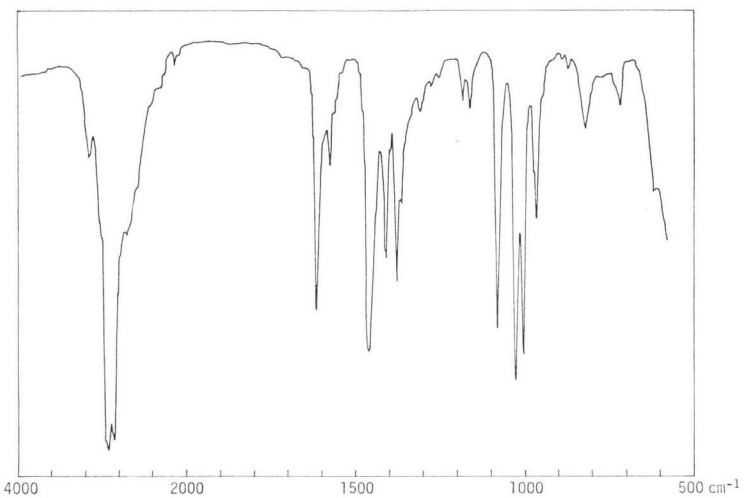
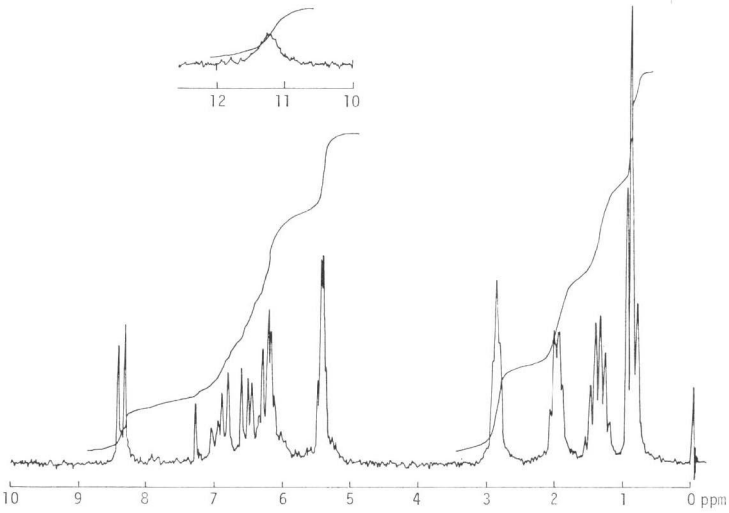


Fig. 2. PMR spectrum of WS-1228 A (**1**) ($CDCl_3$).



2) showed the presence of a hydroxyl function (δ 11.3), *n*-propyl group adjacent to a double bond (δ 0.90, 1.35 and 2.00), a methylene between double bonds (δ 2.88) and a disubstituted isolated double bond (δ 5.45). The spectrum also exhibited the signals at δ 5.9~7.1 (4H, m) due to the conjugated double bond and at δ 8.36 (1H, d, J=8Hz) assigned to an imino olefinic proton.

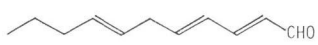
Catalytic hydrogenation of **1** over 10% Pd-C afforded *n*-undecylamine (**3**).

Treatment of **1** with *p*-toluenesulfonic acid in aqueous acetone at room temperature overnight gave the aldehyde (**4**) ($C_{11}H_{16}O$). Its IR spectrum showed absoption bands attributable to the conjugated aldehyde at 1680 and 1640 cm^{-1}. The signals in its PMR spectrum were assigned as follows: δ 0.89 (3H, t, J=6.5 Hz, C_{11}-H), 1.40 (2H, m, C_{10}-H), 2.00 (2H, m, C_9-H), 2.92 (2H, m, C_8-H), 5.45 (2H, m, $C_{7,8}$-H), 6.08 (1H, dd, J=8 and 16 Hz, C_2-H), 6.2~6.45 (2H, m, $C_{4,5}$-H), 7.10 (1H, m, C_3-H) and 9.53 (1H, d, J=8 Hz, CHO). Decoupling experiment using the pseudo-contact shift with $Eu(DPM)_3$ as a shift reagent provided that the diene system conjugated with the aldehyde group had E-E configuration. The configuration of the isolated double bond was confirmed by synthesis of (E,E,E)-2,4,7-undecatrienal (**4**) as described below.

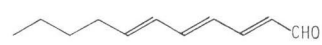
Thus, from these results the structure of WS-1228 A was deduced to be **1**.

WS-1228 B (**2**), yellow needles, had the molecular formula $C_{11}H_{17}N_3O$, established by elemental analysis and mass spectrometry. Its IR spectrum (1593 cm^{-1}) shown in Fig. 3 and UV spectrum (339 nm) suggested the presence of the tetraene oxime moiety. WS-1228 B (**2**) also had a positive color reaction to ferric chloride. The six olefinic protons (δ 5.93~7.40) and the one imino olefinic proton (δ 8.40)

(3)



(4)



(5)

Fig. 3. IR spectrum of WS-1228 B (**2**) (nujol).

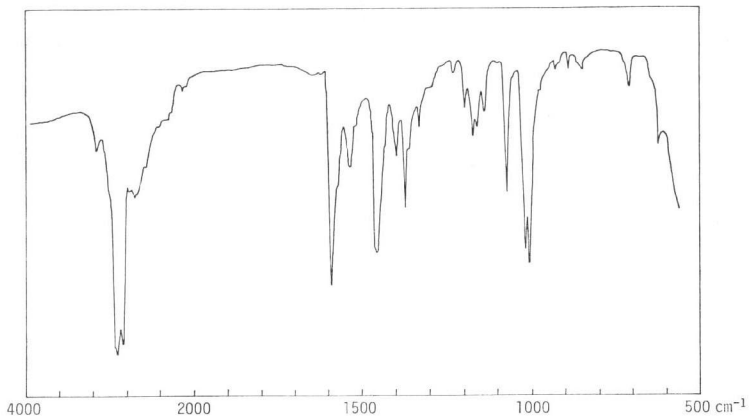
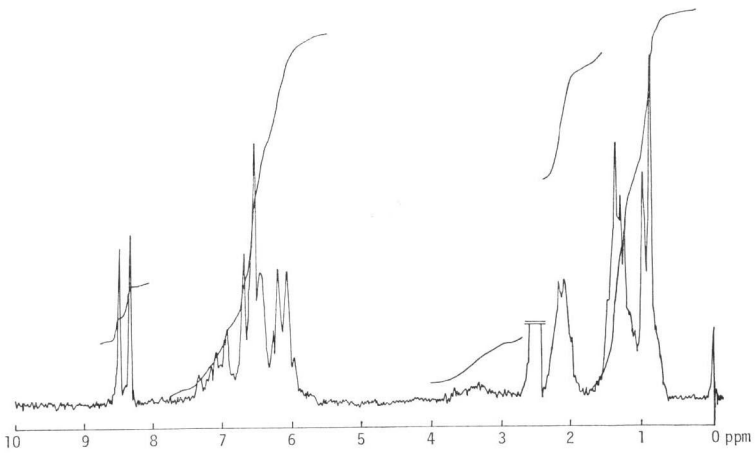


Fig. 4. PMR spectrum of WS-1228 B (**2**) (DMSO-d_6).



were observed in the PMR spectrum shown in Fig. 4. Considering these facts it was deduced that WS-1228 B (**2**) is a positional isomer of WS-1228 A (**1**).

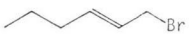
Hydrolysis of **2** with *p*-toluenesulfonic acid in aqueous acetone gave the aldehyde (**5**) ($C_{11}H_{16}O$). Its IR spectrum showed absorption bands attributable to the conjugated aldehyde at 1675 and 1618 cm^{-1}. Comparison of the PMR spectrum of **5** with that of **4** indicated that the signal due to the additional two conjugated olefinic protons appeared in stead of that due to the protons of the isolated double bond, and the signal (δ 2.92) due to the C-6 methylene group in **5** was shifted to the higher field, indicating that the structure of the aldehyde (**5**) was deduced to be 2,4,6-undecatrienal. Decoupling experiment also exhibited that the triene system conjugated with the aldehyde group had E-E-E configuration. It was concluded that WS-1228 B (**2**) also possessed the unique moiety, *N*-hydroxytriazene.

Thus, the structure **2** appeared most plausible for WS-1228 B.

In order to confirm the structures of WS-1228 A (**1**) and B (**2**), synthesis of WS-1228 A (**1**) and the aldehyde (**5**) were undertaken as follows.

The copper catalyzed coupling reaction[2] of *trans*-hexenyl bromide[3] (**6**) with propargyl tetrahydropyranyl ether (**7**) using ethylmagnesium bromide and cuprous cyanide in tetrahydrofuran gave the ether

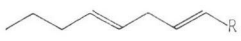
(**8**) in 51.4% yield which was smoothly hydrolyzed to the corresponding alcohol (**9**) with 2% oxalic acid in methanol at room temperature. Reduction of **9** with sodium in liquid ammonia gave nonadienol (**10**) in 71.8% yield which was converted to nonadienal (**11**) with activated manganese dioxide in chloroform. The condensation of nonadienal (**11**) with sodio triethyl phosphonoacetate in benzene[4] afforded selectively ethyl (E,E,E)-2,4,7-undecatrienoate (**12**) as a sole product in 44.6% yield. Reduction of **12** with lithium aluminum hydride in ether followed by oxidation with activated manganese dioxide in chloroform gave (E,E,E)-2,4,7-undecatrienal (**4**) which was identical with the aldehyde (**4**) derived from natural sources. Thus, the carbon skeleton of WS-1228 A (**1**) was prepared in hand, our attention was turned to the construction of the triazene moiety.



(**6**)

(**7**)

(**8**) R = THP
(**9**) R = H



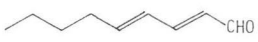
(**10**) R = CH_2OH
(**11**) R = CHO

(**12**)

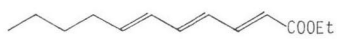
(**13**)

Reaction of the aldehyde (**4**) with hydrazine hydrate in ethanol afforded the unstable hydrazone (**13**). Attempts to effect nitrosation were unsuccessful under a variety of conditions. However, trimethylsilylation of the crude hydrazone (**13**) followed by nitrosation with gaseous dinitrogen trioxide in methylene chloride at −20°C gave WS-1228 A (**1**) in a low yield which was purified by preparative HPLC. All the spectral data of synthetic WS-1228 A (**1**) were superimposable to those of natural WS-1228 A, confirming the structure of **1**. The hypotensive activity of the synthetic compound in anesthetized rat was identical to that of natural WS-1228 A.

Next, in order to confirm the carbon skeleton of WS-1228 B (**2**), synthesis of (E,E,E)-2,4,6-undecatrienal (**5**) were undertaken.



(**14**)



(**15**)

Treatment of commercially available (E,E)-2,4-nonadienal (**14**) with triethyl phosphonoacetate in the presence of sodium hydride in benzene[4] gave the ester (**15**) in 68.8% yield. Reduction of **15** with lithium aluminum hydride in ether followed by oxidation with activated manganese dioxide in chloroform gave (E,E,E)-2,4,6-undecatrienal which was identical with the aldehyde (**5**) derived from natural WS-1228 B (**2**).

To our knowledge, WS-1228 A (**1**) and B (**2**) are the first naturally occurring compounds possessing a triazene group.

Experimental

All melting points were determined with a Yanagimoto microscope hot-stage apparatus and uncorrected. IR spectra were recorded by a Jasco IRA-2 grating spectrophotometer. UV spectra were recorded by a Hitachi Model 200–20 spectrophotometer. PMR were obtained with JEOL-JMN-PMX 60 NMR spectrometer (tetramethyl silane as an internal standard). Mass spectra were determined with a Hitachi RMU-6M spectrometer with a direct, heated inlet system.

WS-1228 A (1) and WS-1228 B (2)

Fermentation and isolation were described in the preceding paper[1].

WS-1228 A (**1**) was recrystallized from ethanol - chloroform. mp 100~102°C (dec.). MS *m/z* 207 (M^+). UV λ_{max}^{MeOH} 300 nm (ε 44,000). IR ν_{max}^{Nujol} 3300~2200, 1612, 1580, 1410, 1080, 1025, 1005 and 975 cm^{-1}. PMR ($CDCl_3$) δ 0.90 (3H, t, *J*=6.5 Hz), 1.35 (2H, m), 2.00 (2H, m), 2.88 (2H, m), 5.45 (2H, m), 5.9~7.1 (4H, m), 8.36 (1H, d, *J*=8 Hz) and 11.3 (1H, br. s, disappeared with D_2O).

Anal. Calcd. for $C_{11}H_{17}N_3O$: C 63.74, H 8.27, N 20.27.
Found: C 63.88, H 8.28, N 20.09.

WS-1228 B (**2**) was recrystallized from ethanol. mp 135~138°C. MS *m/z* 207 (M^+). UV λ_{max}^{MeOH} 339 nm (ε 43,000). IR ν_{max}^{Nujol} 3300~2250, 1593, 1538, 1405, 1078, 1022 and 1010 cm^{-1}. PMR (DMSO-d_6) δ 0.93 (3H, t, *J*=7 Hz), 1.33 (4H, m), 2.15 (2H, m), 5.93~7.40 (6H, m) and 8.40 (1H, d, *J*=9.7 Hz).

Anal. Calcd. for $C_{11}H_{17}N_3O$: C 63.47, H 8.27, N 20.27.
Found: C 63.46, H 8.23, N 20.51.

Hydrogenation of WS-1228 A (1)

1 (100 mg) was hydrogenated in ethanol (20 ml) with 10% Pd-C (50 mg) as catalyst. The usual work-up gave *n*-undecylamine (70 mg) which was identical with the authentic specimen (Suppleir: Nakarai Chemicals, Ltd.).

Acid Hydrolysis of WS-1228 A (1)

A solution of **1** (100 mg) and *p*-toluenesulfonic acid (10 mg) in acetone - water (20 ml: 5 ml) was allowed to stand at room temperature overnight. The usual work-up gave an oily residue which was chromatographed on silica gel with chloroform. Elution with chloroform gave the aldehyde (**4**) (60 mg) as an oil. IR ν_{max}^{film} 2870, 1680, 1640, 1160, 1120, 1010, 990 and 970 cm^{-1}. PMR ($CDCl_3$) δ 0.89 (3H, t, *J*=6.5 Hz), 1.40 (2H, m), 2.00 (2H, m), 2.92 (2H, m), 5.45 (2H, m), 6.08 (1H, dd, *J*=8 and 16 Hz), 6.2~6.45 (2H, m), 7.10 (1H, m) and 9.53 (1H, d, *J*=8 Hz). MS *m/z* 164 (M^+).

Acid Hydrolysis of WS-1228 B (2)

WS-1228 B (**2**) (100 mg) was subjected to the same reaction sequence as mentioned above to give the aldehyde (**5**) (55 mg) as an oil. IR ν_{max}^{film} 2730, 1675, 1618, 1165, 1125, 1015 and 995 cm^{-1}. PMR ($CDCl_3$) δ 0.91 (3H, t, *J*=7.0 Hz), 1.4 (4H, m), 2.17 (2H, m), 5.8~6.8 (5H, m), 7.10 (1H, dd, *J*=15 and 7.5 Hz) and 9.50 (1H, d, *J*=7.5 Hz). MS *m/z* 164 (M^+).

1-(2-Tetrahydropyranyloxy)-5-nonen-2-yne (8)

To a solution of ethylmagnesium bromide (0.36 mole) [prepared from magnesium (8.6 g) and ethyl bromide (39 g)] in dry tetrahydrofuran (100 ml) was added a solution of tetrahydropyranyloxypropyne (50 g) in dry tetrahydrofuran (75 ml) at 0°C over a period of 15 minutes under nitrogen. To this solution was added cuprous cyanide (20 mg) and the resulting solution was stirred at room temperature for 30 minutes at which time a solution of hexenyl bromide (58 g) in dry tetrahydrofuran (75 ml) was added over a period of 15 minutes under cooling. The whole was stirred at room temperature for 30 minutes, and concentrated to dryness under reduced pressure to give a residue which was diluted with dilute hydrochloric acid and extracted with ether (150 ml×2). The ether solution was washed with water, dried and evaporated under reduced pressure to afford an oily residue which was distilled under reduced pressure to give 1-(2-tetrahydropyranyloxy)-5-nonen-2-yne (**8**) (58 g) as a colorless oil. bp 142~144°C/6.5 mm. IR ν_{max}^{film} 2950, 2260 and 1110 cm^{-1}. PMR ($CDCl_3$) δ 0.90 (3H, t, *J*=7 Hz), 1.13~2.27 (10H, m), 3.00 (2H, m), 3.33~4.13 (2H, m), 4.30 (2H, s), 4.85 (1H, s) and 5.17~6.00 (2H, m).

5-Nonen-2-yn-1-ol (**9**)

A solution of 1-(2-tetrahydropyranyloxy)-5-nonen-2-yne (**8**) (61.5 g) and 2% oxalic acid (250 ml) in methanol (250 ml) was refluxed for 2 hours. Removal of the solvent under reduced pressure gave a residue which was taken up in ether (200 ml). The ether solution was washed with water, dried and evaporated under reduced pressure to give an oily residue which was distilled to afford 5-nonen-2-yn-1-ol (**9**) (37 g) as a colorless oil. bp 89~90°C/3.3 mm. IR ν_{max}^{film} 3400, 2930, 2300, 2250, 1080 and 970 cm^{-1}. PMR ($CDCl_3$) δ 0.90 (3H, t, J=7 Hz), 1.38 (2H, m), 2.00 (2H, m), 2.93 (2H, m), 4.26 (2H, s), 5.27 (1H, m) and 5.79 (1H, m). MS m/z 138 (M^+).

2,5-Nonadien-1-ol (**10**)

A solution of 5-nonen-2-yn-1-ol (**9**) (21 g) in ether (100 ml) was added to a mixture of sodium (11 g) in liquid ammonia (100 ml) and ammonium sulfate (105 g), and the mixture was stirred for 6 hours. An excess of ammonium chloride was added, and the ammonia was allowed to evaporate. The ether solution was separated, washed with water, dried, and evaporated. Distillation gave 2,5-nonadien-1-ol (**10**) (15.3 g) as a colorless oil. bp 89~90°C/10.5 mm. IR ν_{max}^{film} 3300, 2950 and 970 cm^{-1}. PMR ($CDCl_3$) δ 0.90 (3H, t, J=7 Hz), 1.40 (2H, m), 2.00 (2H, m), 2.77 (2H, m), 4.12 (2H, m), 5.47 (2H, m) and 5.70 (2H, m). MS m/z 122 (M−18).

2,5-Nonadien-1-al (**11**)

A suspended solution of 2,5-nonadien-1-ol (**10**) (15 g) and activated manganese dioxide (150 g) in chloroform (1,500 ml) was stirred at room temperature for 20 hours. The usual work-up gave a residue which was chromatographed on silica gel in chloroform. Elution with chloroform gave the aldehyde (**11**) (7 g) as a colorless oil. IR ν_{max}^{film} 2950, 1685, 1630 and 970 cm^{-1}. PMR ($CDCl_3$) δ 0.90 (3H, t, J=7 Hz), 1.40 (2H, m), 2.05 (2H, m), 3.07 (2H, m), 5.53 (2H, m), 6.13 (1H, dd, J=8 and 16 Hz), 6.90 (1H, dt, J=6 and 16 Hz) and 9.57 (1H, d, J=8 Hz). MS m/z 138 (M^+).

Ethyl (E,E,E)-2,4,7-undecatrienoate (**12**)

To a suspension of sodium hydride (50%: 2.68 g) in dry benzene (40 ml) was added dropwise triethyl phosphonoacetate (11.5 g) with stirring at room temperature under nitrogen atmosphere, and the mixture was stirred for 2 hours. To this solution was added dropwise a solution of the aldehyde (**11**) (7 g) in benzene (40 ml), and the whole was stirred at room temperature for 30 minutes and washed with water. The aqueous layer was extracted with ether (70 ml). The combined organic layer was washed with water, dried and evaporated under reduced pressure to give an yellow oily residue which was chromatographed on silica gel in chloroform. Elution with chloroform gave the ester (**12**) (4.7 g) as a colorless oil. IR ν_{max}^{film} 2950, 1710, 1640 and 1620 cm^{-1}. PMR ($CDCl_3$) δ 0.90 (3H, t, J=6 Hz), 1.28 (3H, t, J=7 Hz), 1.40 (2H, m), 2.00 (2H, m), 2.86 (2H, m), 4.18 (2H, q, J=7 Hz), 5.42 (2H, m), 5.63~6.43 (3H, m) and 7.03~7.50 (1H, m). MS m/z 208 (M^+).

(E,E,E)-2,4,7-Undecatrien-1-al (**4**)

Lithium aluminum hydride (1 g) was added in portions to a solution of the ester (**12**) (4.7 g) in dry ether (200 ml) with stirring at 0°C, and the mixture was stirred at the same temperature for 1 hour. The usual work-up gave (E,E,E)-2,4,7-undecatrien-1-ol (3.4 g). IR ν_{max}^{film} 3350, 2950 and 990 cm^{-1}. PMR ($CDCl_3$) δ 0.90 (3H, s, J=7 Hz), 1.40 (2H, m), 2.01 (2H, m), 2.82 (2H, m), 4.18 (2H, d, J=6 Hz) and 5.33~6.60 (6H, m). MS m/z 148 (M−18).

A suspended solution of (E,E,E)-2,4,7-undecatrien-1-ol (3.4 g) and activated manganese dioxide (30 g) in chloroform (300 ml) was stirred at room temperature for 60 hours. The usual work-up gave an yellow oily residue which was chromatographed on silica gel in chloroform. Elution with chloroform gave the aldehyde (**4**) (2 g) as a colorless oil. IR ν_{max}^{film} 2950, 1680, 1640, 1120 and 990 cm^{-1}. PMR ($CDCl_3$) δ 0.89 (3H, t, J=6.5 Hz), 1.40 (2H, m), 2.00 (2H, m), 2.92 (2H, m), 5.45 (2H, m), 6.08 (1H, dd, J=8 and 16 Hz), 6.2~6.45 (2H, m), 7.10 (1H, m) and 9.53 (1H, d, J=8 Hz). MS m/z 164 (M^+).

WS-1228 A (**1**)

To a solution of hydrazine hydrate (10 ml) in ethanol (10 ml) was added dropwise the aldehyde (**4**) (2 g) in ethanol (10 ml) with stirring at room temperature, and the mixture was stirred at the same temperature for 30 minutes. Removal of the solvent under reduced pressure gave a residue which was

taken up in ether (30 ml). The ether solution was washed with water and concentrated to dryness under reduced pressure to give the hydrazone (**13**) (1.5 g).

To a solution of the crude hydrazone (**13**) (1.5 g) and triethylamine (1.25 g) in dry ether (20 ml) was added dropwise trimethylsilyl chloride (1.33 g) with stirring under cooling. The whole was stirred at room temperature for 2 hours. Precipitated triethylamine hydrochloride was filtered off and the filtrate was concentrated to dryness under reduced pressure to leave a residue which was taken up in methylene chloride (20 ml). The methylene chloride solution was treated with dinitrogen trioxide, prepared from sodium nitrite (5 g) and fuming nitric acid (3 ml), at −20°C for 3 hours. The resulting solution was washed with water, dried and evaporated under reduced pressure to give a residue which was chromatographed on silica gel in chloroform. Elution with chloroform, and evaporation of the appropriate fractions gave an oily residue, further purified by preparative HPLC to yield WS-1228 A (**1**) (10 mg) which was identical in all respects with WS-1228 A from natural sources. IR ν_{max}^{Nujol} 3300~2200, 1612 and 1580 cm^{-1}.

Ethyl (E,E,E)-2,4,6-undecatrienoate (**15**)

2,4-Nonadien-1-al (**14**) (8.2 g) was subjected to the same reaction sequence as for preparation of ethyl (E,E,E)-2,4,7-undecatrienoate (**12**) to give ethyl (E,E,E)-2,4,6-undecatrienoate (**15**) (8.4 g) as a colorless oil. IR $\nu_{max}^{CHCl_3}$ 1700, 1618 and 1005 cm^{-1}. PMR ($CDCl_3$) δ 0.90 (3H, t, J=7 Hz), 1.27 (3H, t, J=7 Hz), 1.13~1.63 (4H, m), 2.15 (2H, m), 4.22 (2H, q, J=7 Hz), 5.67~6.77 (5H, m) and 7.07~7.57 (1H, m).

(E,E,E)-2,4,6-Undecatrien-1-al (**5**)

Ethyl (E,E,E)-2,4,6-undecatrienoate (**15**) (8.4 g) was treated with lithium aluminum hydride (1.6 g) in the same manner as mentioned for ethyl (E,E,E)-2,4,7-undecatrienoate (**12**) to give (E,E,E)-2,4,6-undecatrien-1-ol (6.2 g). IR $\nu_{max}^{CHCl_3}$ 3600, 2920 and 1000 cm^{-1}. PMR ($CDCl_3$) δ 0.90 (3H, t, J=7 Hz), 1.13~1.60 (4H, m), 1.83~2.57 (2H, m), 4.18 (2H, d, J=6 Hz) and 5.33~6.67 (6H, m).

(E,E,E)-2,4,6-Undecatrien-1-ol (6.2 g) was treated with activated manganese dioxide (50 g) in chloroform (300 ml) in the same way as for (E,E,E)-2,4,7-undecatrien-1-ol to give (E,E,E)-2,4,6-undecatrien-1-al (**5**) (5.5 g) as a colorless oil. IR $\nu_{max}^{CHCl_3}$ 1675 and 1618 cm^{-1}. PMR ($CDCl_3$) δ 0.91 (3H, t, J=7 Hz), 1.40 (4H, m), 2.17 (2H, m), 5.8~6.8 (5H, m), 7.10 (1H, dd, J=15 and 7.5 Hz) and 9.50 (1H, d, J=7.5 Hz).

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