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Syntheses and Antimicrobial Activities of Fomecins A and B, Asperugin, and Related Compounds

KUNIO HAYASHI, KATSUYA TOKURA, KEI OKABE,* KIYOE YAMAMOTO, and KATSUYA TAWARA

Shionogi Research Laboratories, Shionogi and Co., Ltd., Fukushima-ku, Osaka, 553 Japan

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Fomecins A and B, phenolic antibiotics produced by the basidiomycete *Fomes juniperinus* SCHRENK, and asperugin, a metabolite of *Aspergillus rugulosus*, were synthesized from gallic acid. Various congeners were also prepared. Their antibacterial and antifungal activities were tested *in vitro* and some of the products were found to exhibit moderate antifungal activities.

Keywords——fomecin A; fomecin B; asperugin; phenol; fungal metabolite; antimicrobial activity

Fomecins A and B were first isolated from culture liquid of *Fomes juniperinus* by Anchel *et al.*[1a] and were assigned the structures **1** and **2**, respectively.[1b] They have been shown to exhibit rather broad antibacterial and antiviral activities.[1a] Asperugin (**3**), isolated from the culture fluid of a mutant strain of *Aspergillus rugulosus*, was characterized on the basis of degradation experiments and spectroscopic evidence.[2] Besides these three compounds, many closely related penta- or hexa-substituted monobenzenoids, such as asperugin B (**4**),[3] flavipin (**5**),[4] gladiolic acid (**6**),[5] scutigeral (**7**),[6] and zinniol (**8**),[7] have been reported as fungal metabolites. Some of them are known to show antibacterial or antifungal activities. Although a report of synthesis of **6** appeared in 1956,[8] only recently have the syntheses of **2**,[9] **5**,[10] **7**,[6] and **8**[11] been reported. We synthesized **1** and **2** in the course of a study aimed at finding polyhydric phenols with useful antifungal activities and published the results in a preliminary communication.[12] We wish to report here the details of the work, together with the syntheses of **3** and its congeners, and the antimicrobial activities of the compounds synthesized.

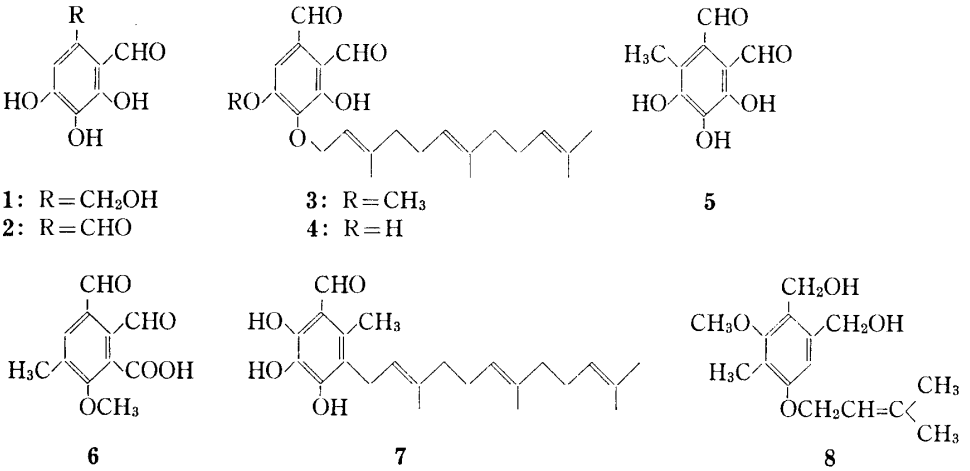


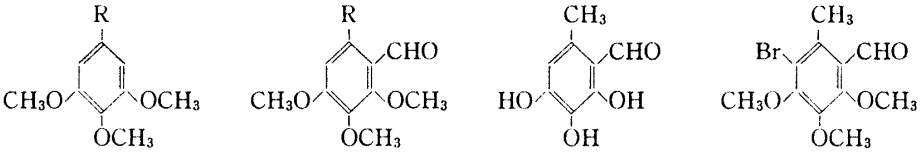
Chart 1

Syntheses of Fomecin A (1) and Fomecin B (2)

The starting material chosen was commercially available gallic acid. The three vicinal hydroxyl groups are susceptible to air oxidation, especially under basic conditions and need to

be protected by groups removable preferably under neutral or acidic conditions. While various be protected by groups removable preferably under neutral or acidic conditions. While various protecting groups can be used, methyl ethers were employed here because of their ease of introduction and anticipated smooth cleavage in the later stage of synthesis.

Gallic acid was transformed into **11** *via* methyl 3,4,5-trimethoxybenzoate (**9**) and 3,4,5-trimethoxybenzyl alcohol (**10**) by a known procedure.[13] Compound **11** was formylated using Vilsmeier reagent and gave **12**.[14] Deprotection of **12** with boron tribromide (BBr_3) afforded **13**, which showed mp identical to the reported value.[15] In the reaction of **12** with *N*-bromosuccinimide under irradiation, only nuclear bromination took place to give **15**, and none of the desired benzyl bromide (**14**) was isolated.



9: R=$COOCH_3$
10: R=CH_2OH
11: R=CH_3

12: R=CH_3
14: R=CH_2Br

13

15

Chart 2

Formylation of **10** with dichloromethyl methyl ether and a catalytic amount of titanium tetrachloride ($TiCl_4$) yielded 6-chloromethyl-2,3,4-trimethoxybenzaldehyde (**16**). The presence of a chlorine atom in **16** was confirmed by a singlet at δ 5.05 in the ^1H NMR spectrum, peaks at *m/z* 246 (M+2), 244 (M^+), and 209 in the mass spectrum (MS), and microanalysis. The methyl ether (**17**) derived from **10** with methyl iodide and sodium hydride also gave rise to **16** on formylation using dichloromethyl methyl ether. Treatment of **16** with silver nitrate in methanol (MeOH) yielded the methyl ether (**18**), which was cleaved with BBr_3 to afford the benzyl bromide (**19**). The structure of **19** was confirmed by ^1H NMR spectroscopy, MS, and microanalysis. Compound **19** was converted easily to **20** on treatment with MeOH. Acetylation of **20** with acetic anhydride (Ac_2O) and pyridine, followed by ether–ester exchange with boron trifluoride etherate and Ac_2O afforded the benzyl acetate (**22**), which is structurally the tetraacetate of **1**. Alkaline hydrolysis of **22** to **1** was not successful. Attempts to convert the benzyl halides (**16** or **19**) into benzyl alcohols under various conditions (AgOAc, $AgNO_3$, or Cu_2O) gave only complex products.

The oxidative approach was next tried. Among the oxidizing agents generally used for the conversion of halomethyl compounds into aldehydes (such as sodium dichromate, lead nitrate, hexamethylenetetramine), selenium dioxide (SeO_2) was our choice. Oxidation of **16** with SeO_2 in refluxing dioxane gave **23**, the structure of which was confirmed by the presence of two singlets due to formyl protons at δ 10.50 and 10.47 and three singlets due to methoxyl groups at δ 4.03, 3.98, and 3.97 in the ^1H NMR spectrum. This SeO_2 oxidation also gave the lactol (**24**) as a by-product. Treatment of **24** with MeOH and *p*-toluenesulfonic acid gave **25**, which was identified by comparison of mp, IR, and NMR with those of the sample derived from **9** with dichloromethyl methyl ether. Cleavage of **23** with BBr_3 afforded **2**, which was also obtained by the SeO_2 oxidation of **19**. Compound **2** could be reconverted to **23** by reaction with dimethyl sulfate. Compound **2** had physical properties (mp, and UV, IR, and NMR spectra) identical with those reported for natural fomecin B[1b] and for **2** obtained by the degradation of natural asperugin B.[3]

The non-chelated formyl group was selectively reduced by catalytic hydrogenation at 3 atm using platinum oxide as a catalyst. This hydrogenation gave a mixture of **1** and unchanged **2**. Chromatographic separation of the mixture on silica gel, Lichrosorb Diol, or Sephadex LH-20 resulted in poor separation or complete loss of the material. The separa-

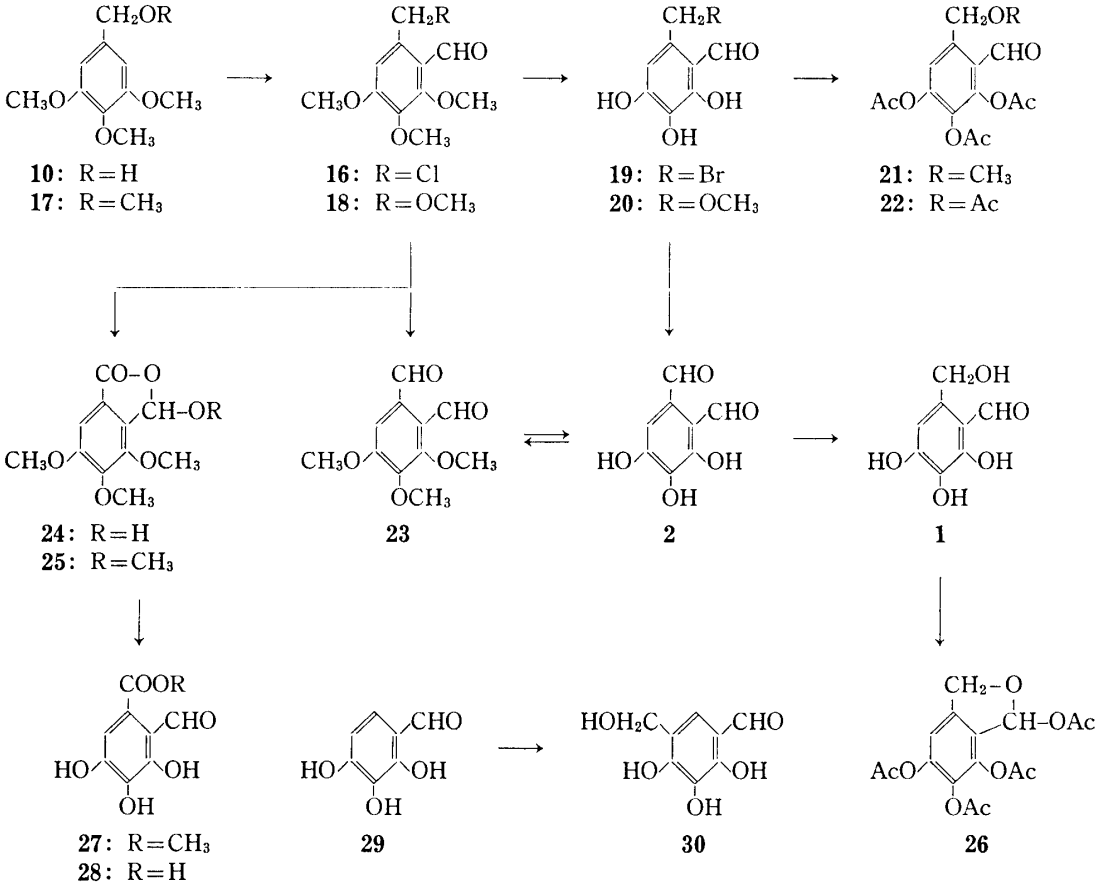


Chart 3

tion of **1** from **2** was achieved by countercurrent distribution.[1a] Compound **1** was proved to be identical with an authentic sample of natural fomecin A (mp, and UV, IR, ^{1}H NMR, ^{13}C NMR, and mass spectra). Furthermore, on acetylation with Ac_2O and pyridine, synthetic **1** gave a tetraacetate (**26**) which was identical with that derived from the natural material.[1b] Compound **26** was clearly different from the tetraacetate **22** described above. The ^{1}H NMR spectrum of **22** showed a singlet due to methylene protons at δ 5.45 and a singlet due to a formyl proton at δ 10.18, whereas that of **26** exhibited no signal due to a formyl proton, but ABX-type signals at δ 7.37 (X, dd, J=1.5 and 1.0 Hz), δ 5.29 (A, dd, J=13.0 and 1.0 Hz) and δ 5.09 (B, dd, J=13.0 and 1.5 Hz); broadening of the signals A and B indicated further couplings with the aromatic proton. This observation suggests the bicyclic structure of **26**.

A few related compounds were prepared for comparison of antimicrobial activities. Reaction of **25** with BBr_3 followed by treatment with MeOH yielded the methylester (**27**), which, upon hydrolysis with 10% sulfuric acid, gave the carboxylic acid (**28**). 2,3,4-Trihydroxybenzaldehyde (**29**)[16] prepared from pyrogallol was hydroxymethylated to afford **30**.

Syntheses of Asperugin and Its Congeners

Compound **2** was methylated in 5% aqueous sodium tetraborate solution using dimethyl sulfate and sodium hydroxide[17] to obtain the monomethyl ether (**31**). The physical properties of **31** were consistent with those of the hydrolysis product of asperugin reported by Ballantine *et al.*[2] Treatment of **31** with farnesyl bromide in the presence of sodium iodide and potassium carbonate afforded a product (**3**) with IR and ^{1}H NMR spectroscopic data that were found to be identical with those reported for asperugin.[2]

Some congeners of **3** were prepared in order to test the antimicrobial activities. Reaction of **31** with prenyl bromide or geranyl chloride in similar ways yielded **32a—d**. Catalytic

TABLE I. Congeners of Asperugin (3)

Compd No.	Substituent[a]		Yield %	mp °C	Formula	IR $\nu_{max}^{CHCl_3}$ cm^{-1}			Selected ^{1}H NMR ($CDCl_3$) δ	
	R_1	R_2				OH	C=O	CHO	ArH	OH
3	H	Far	15	Oil	$C_{24}H_{32}O_5$[b]	3500—3300(br)	1690, 1635	10.82, 10.08	7.20(s)	12.42
32a	H	Ger	23	Oil	$C_{19}H_{24}O_5$[b]	3500—2400(br)	1690, 1660	10.83, 10.12	7.03(s)	12.43
32b	Ger	Ger	9	Oil	$C_{29}H_{40}O_5$[b]		1675	10.53, 10.45	7.25(s)	
32c	H	Pre	13	Oil	$C_{14}H_{16}O_5$[b]	3600—2400(br)	1690, 1635	10.85, 10.08	7.05(s)	12.45
32d	Pre	Pre	19	58—61	$C_{19}H_{24}O_5$[c]		1680	10.50, 10.42	7.28(s)	
36a	H	All	18	Oil	$C_{11}H_{12}O_4$[c]	3400—2800(br)	1640	9.73	7.27(d, J=8), 6.58(d, J=8)	11.13
36b	All	H	19	78	$C_{11}H_{12}O_4$[c]	3530	1680	10.27	7.43(d, J=8), 6.73(d, J=8)	5.78
36c	All	All	23	Oil	$C_{14}H_{16}O_4$[c]		1680	10.24	7.57(d, J=8), 6.73(d, J=8)	
36d	H	Pre	42	Oil	$C_{13}H_{16}O_4$[c]	3600—2400(br)	1640	9.77	7.29(d, J=8), 6.59(d, J=8)	11.12
36e	Pre	H	21	89—91	$C_{13}H_{16}O_4$[c]	3525	1675	10.05	7.42(d, J=8), 6.73(d, J=8)	5.72
36f	Pre	Pre	11	Oil	$C_{18}H_{24}O_4$[c]		1680	10.15	7.53(d, J=8), 6.68(d, J=8)	
36g	H	Ger	29	Oil	$C_{18}H_{24}O_4$[b]	3600—2400(br)	1640	9.75	7.07(d, J=8), 6.57(d, J=8)	11.10
36h	Ger	H	53	75—77	$C_{18}H_{24}O_4$[c]	3530	1678	10.08	7.42(d, J=8), 6.73(d, J=8)	5.80
36i	Ger	Ger	4	Oil	$C_{28}H_{40}O_4$[b]		1670	10.25	7.60(d, J=8), 6.75(d, J=8)	
36j	H	Far	12	Oil	$C_{23}H_{32}O_4$[b]	3600—2400(br)	1630	9.73	7.23(d, J=8), 6.62(d, J=8)	11.08
36k	Far	H	59	64—66	$C_{23}H_{32}O_4$[c]	3520	1670	10.23	7.42(d, J=8), 6.73(d, J=8)	5.83
36l	Far	Far	2	Oil	$C_{38}H_{56}O_4$[b]		1670	10.23	7.52(d, J=8), 6.68(d, J=8)	

a) Abbreviations: All, allyl; Far, farnesyl; Ger, geranyl; Pre, prenyl.

b) Formulae were confirmed by MS [M^+ or $(M+1)^+$ peak].

c) Analytical values were within ±0.4% of the calculated values.

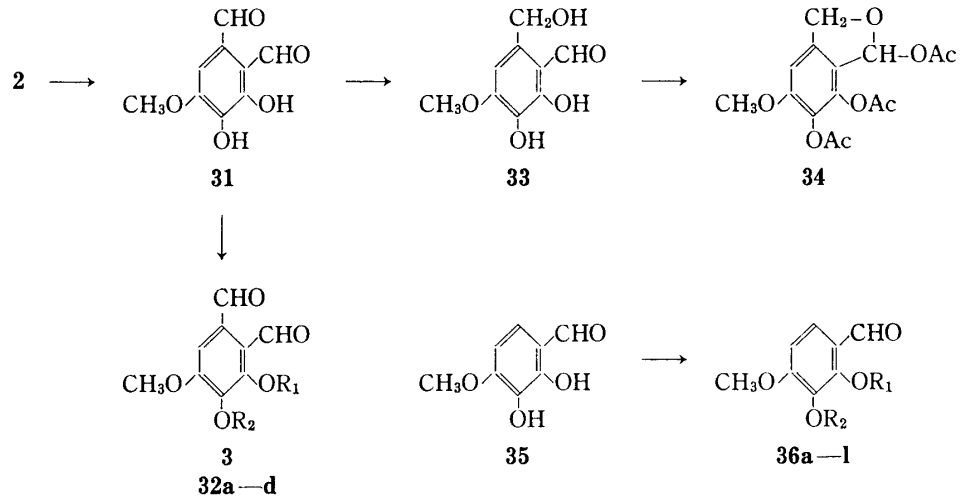


Chart 4

hydrogenation of **31** over palladium on carbon took place smoothly to afford **33**, in contrast to the case of **2**. Acetylation of **33** with Ac_2O and pyridine yielded the cyclized triacetate (**34**). None of the expected prenyl ethers was isolated in the reaction of **33** with prenyl bromide. Ethers (**36a—1**) missing one of the two formyl groups in **32** were also prepared similarly, starting from **29**, *via* **35**. These congeners of **3** are listed in Table I. Their structures were confirmed by observation of the ^1H NMR signals of C-methyl, formyl, and chelated hydroxyl groups, and by the carbonyl absorption in IR spectra.

Antimicrobial Activities

The antimicrobial activities of **1**, **2**, **3** and related compounds were tested *in vitro* against Gram-positive and Gram-negative bacteria and fungi. Compounds **17**, **22**, **26**, **32d**, **33**, **36b**, **36d**, **36i**, **36k**, and **36l** were inactive. Other compounds showed weak activity. Among them, compounds **32a** and **35** were active at 6.2 μg/ml against *Candida albicans*. Compounds **29** and **36g** were active at 6.2 and 12.5 μg/ml, respectively, against *Trichophyton asteroides*. However, no structure-activity relationships could be found.

Experimental

Melting points were determined with a Yanagimoto micro melting point apparatus and are uncorrected. Column chromatography was carried out on silica gel 60 (0.063—0.20 mm, Merck). *Rf* values were obtained from thin-layer chromatograms (TLC) on Merck Kieselgel 60 F_{254} (0.25 mm) plates using one of four solvent systems: system A, benzene–ethyl acetate (AcOEt) (9: 1 v/v); system B, $CHCl_3$–MeOH (9: 1 v/v); system C, $CHCl_3$–MeOH–acetic acid (AcOH) (90: 10: 3 v/v); system D, benzene–dioxane–AcOH (90: 25: 4 v/v). Infrared spectra were obtained with a JASCO DS-403G infrared spectrometer or a Hitachi 215 grating infrared spectrometer. Ultraviolet spectra were measured with a Hitachi 323 spectrophotometer. 60-MHz ^1H NMR spectra were measured with a Varian EM-360L, NV-14, or T-60A spectrometer. The 15.087-MHz ^1H complete-decoupled ^{13}C FT NMR spectra were determined in DMSO-d_6 with a Varian NV-14 FT NMR spectrometer. Chemical shifts were expressed as δ (ppm downfield from the internal tetramethylsilane signal). Mass spectra were taken with a Hitachi RMU-6E mass spectrometer.

2,3,4-Trihydroxy-6-methylbenzaldehyde (13)——BBr_3 (1 M in CH_2Cl_2, 5 ml) was added slowly to a solution of **12**[14] (210 mg) in CH_2Cl_2 (10 ml) at −75°C. The mixture was kept at this temperature for 40 min and then allowed to warm up to room temperature and stirred for 18 h. An excess of MeOH (5 ml) was added and the solution was concentrated *in vacuo*, affording a solid. Recrystallization of the residue from benzene afforded **13** (56 mg, 33%), mp 184—185°C (lit.[15] mp 182—183°C). *Anal.* Calcd for $C_8H_8O_4$: C, 57.14; H, 4.80. Found: C, 57.12; H, 4.57. *Rf*: 0.26 (B). IR ν_{max}^{Nujol} cm^{-1}: 1600 (chelated C=O). ^1H NMR (acetone-d_6) δ: 12.42 (1H, s, chelated OH), 10.07 (1H, s, CHO), 8.13 (2H, br, 2 × OH), 6.32 (1H, s, ArH), 2.47 (3H, s, CH_3).

5-Bromo-2,3,4-trimethoxy-6-methylbenzaldehyde (15)——A mixture of **12** (50 mg), *N*-bromosuccinimide (45 mg), and carbon tetrachloride (CCl_4) (1 ml) was stirred at 0°C under irradiation (300 W) for 1 h. The mixture was filtered and concentrated under reduced pressure. Crystallization of the residue from cyclo-

TABLE II. Antimicrobial Activities of Fomecins A and B, Asperugin, and Related Compounds

Microorganism	MIC (μg/ml)																			
	1	**2**	**3**	**13**	**19**	**20**	**23**	**29**	**30**	**31**	**32a**	**32b**	**32c**	**35**	**36a**	**36c**	**36e**	**36f**	**36g**	**36h**	**36j**
Candida albicans M-9	>100	50	>100	>100	>100	>100	50	50	>100	25	6.2	>100	12.5	6.2	50	>100	100	>100	>100	>100	>100
Aspergillus fumigatus MA	>100	50	>100	>100	>100	>100	100	>100	>100	50	100	>100	>100	100	50	>100	100	>100	>100	>100	>100
Trichophyton asteroides	100	100	100	50	100	50	100	6.2	50	100	100	>100	100	50	100	50	50	50	12.5	25	100
Staphylococcus aureus 077	25	>100	>100	50	50	25	50	25	50	100	>100	>100	>100	50	>100	>100	>100	>100	>100	>100	>100
Streptococcus pyogenes C-203	100	>100	50	100	100	100	100	100	50	100	50	12.5	>100	>100	>100	100	>100	>100	12.5	25	12.5
Escherichia coli 0208	100	>100	>100	>100	>100	>100	50	100	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100
Klebsiella pneumoniae Shionogi	50	>100	>100	50	100	50	100	50	50	100	>100	>100	>100	50	>100	>100	>100	>100	>100	>100	>100
Pseudomonas aeruginosa PS-24	50	>100	>100	50	100	50	50	50	100	100	>100	>100	>100	100	>100	>100	>100	>100	>100	>100	>100

MIC's were determined by the agar dilution method.

hexane gave **15** (57 mg, 82%), mp 75°C. *Anal.* Calcd for $C_{11}H_{13}BrO_4$: C, 45.69; H, 4.53; Br, 27.64. Found: C, 45.88; H, 4.55; Br, 27.45. *Rf*: 0.61 (A). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 1690 (CHO). ^{1}H NMR ($CDCl_3$) δ: 10.37 (1H, s, CHO), 3.97 (6H, s, 2×OCH_3), 3.90 (3H, s, OCH_3), 2.63 (3H, s, $ArCH_3$).

3,4,5-Trimethoxybenzyl Methyl Ether (17)——NaH (765 mg) was added to a solution of **10** (5.4 g) and methyl iodide (5.1 g) in THF (40 ml) over a period of 15 min. More methyl iodide (1.5 g) was added, and the solution was stirred at room temperature for 2 h. The insoluble material was filtered off and washed with THF (5 ml). The combined filtrate and washings were concentrated. Distillation of the resulting oil gave **17** (5.72 g, 99%), bp 110—111°C/1 mmHg, as a viscous oil. *Anal.* Calcd for $C_{11}H_{16}O_4$: C, 62.25; H, 7.60. Found: C, 61.83; H, 7.77. *Rf*: 0.36 (A). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 1595. ^{1}H NMR ($CDCl_3$) δ: 6.57 (2H, s, ArH), 4.42 (2H, s, CH_2O), 3.85 (9H, s, 3×OCH_3), 3.40 (3H, s, $CH_2OC\underline{H}_3$).

6-Chloromethyl-2,3,4-trimethoxybenzaldehyde (16)——(a) A solution of dichloromethyl methyl ether (18 ml) in CH_2Cl_2 (18 ml) was added with stirring to a solution of **10** (3.96 g) and $TiCl_4$ (10 ml) in CH_2Cl_2 (100 ml) over a period of 40 min. The mixture was stirred for 1 h at 0°C and then for 2 h at room temperature. Next, the mixture was treated with cold dilute HCl and worked up in the usual way. Crystallization of the crude product from cyclohexane gave **16** (2.83 g, 58%) as colorless prisms, mp 86°C. *Anal.* Calcd for $C_{11}H_{13}ClO_4$: C, 53.99; H, 5.36; Cl, 14.49. Found: C, 53.94; H, 5.38; Cl, 14.75. *Rf*: 0.51 (A). IR ν_{max}^{Nujol} cm^{-1}: 1673 (CHO). ^{1}H NMR ($CDCl_3$) δ: 10.45 (1H, s, CHO), 6.95 (1H, s, ArH), 5.05 (2H, s, CH_2Cl), 4.02, 3.97, 3.90 (3×3H, s, OCH_3). ^{13}C NMR ($CDCl_3$) δc: 190.5 (CHO), 158.5, 158.2, 141.5, 135.7, 120.5, 109.5 (6×aromatic C), 62.5, 61.0, 56.2 (3×OCH_3), 44.3 (CH_2Cl). MS *m/z* (relative intensity, %): 246 (30), 244 (100, M^+), 209 (96), 193 (39), 191 (30), 165 (30).

(b) Formylation of **17** (424 mg) as described under method (a) gave a product (342 mg, 70%), mp 86°C, which was identical (mixed mp and IR and ^{1}H NMR spectra) with the material prepared by method (a).

2,3,4-Trimethoxy-6-methoxymethylbenzaldehyde (18)——A solution of **16** (1.43 g) and $AgNO_3$ (1.19 g) in MeOH (35 ml) was stirred under reflux for 1 h. After addition of $CHCl_3$, the voluminous white precipitate formed during the reaction was filtered off. The filtrate was washed with water and concentrated under reduced pressure. The crude product was chromatographed over a column of silica gel (50 g) with benzene–AcOEt (95: 5) as the eluent, to give **18** (1.3 g, 92%), which formed colorless needles from hexane, mp 77°C. *Anal.* Calcd for $C_{12}H_{16}O_5$: C, 59.99; H, 6.71. Found: C, 59.81; H, 6.69. *Rf*: 0.35 (A). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 1670 (CHO). ^{1}H NMR ($CDCl_3$) δ: 10.38 (1H, s, CHO), 7.10 (1H, s, ArH), 4.82 (2H, s, CH_2O), 4.00, 3.97, 3.88 (3×3H, s, OCH_3), 3.52 (3H, s, $CH_2OC\underline{H}_3$).

6-Bromomethyl-2,3,4-trihydroxybenzaldehyde (19)——BBr_3 (1 M in CH_2Cl_2, 6 ml) was added slowly to a solution of **18** (240 mg) in CH_2Cl_2 (10 ml) at −70°C. The mixture was kept at this temperature for 1 h and then allowed to warm up to room temperature. After being stirred for 17 h at room temperature the mixture was poured onto ice. The precipitate was collected, washed with water, and dried, giving **19** (210 mg, 85%) as a yellow powder, which showed no melting point but charred gradually above 150°C. *Anal.* Calcd for $C_8H_7BrO_4$: C, 38.89; H, 2.86; Br, 32.35. Found: C, 38.85; H, 3.01; Br, 33.24. IR ν_{max}^{Nujol} cm^{-1}: 3640, 3140 (OH), 1630 (CHO). ^{1}H NMR (DMSO-d_6) δ: 10.28 (1H, s, CHO), *ca.* 7.17 (3H, br, 3×OH), 6.60 (1H, s, ArH), 4.98 (2H, s, CH_2Br). MS *m/z* (%): 248 (4), 246 (2, M^+), 167 (50), 166 (100), 137 (21), 82 (49), 80 (50).

2,3,4-Trihydroxy-6-methoxymethylbenzaldehyde (20)——BBr_3 (1 M in CH_2Cl_2, 6 ml) was added slowly to a solution of **18** (240 mg) in CH_2Cl_2 (10 ml) at −75°C. The mixture was kept at this temperature for 1 h and then allowed to warm up to room temperature. It was stirred for 17 h, then excess MeOH (6 ml) was added and the solution was concentrated to afford a white solid. Recrystallization from EtOH–H_2O afforded **20** (105 mg, 53%) as colorless needles, mp 210—212°C (dec.). *Anal.* Calcd for $C_9H_{10}O_5$: C, 54.54; H, 5.09. Found: C, 54.17; H, 5.10. *Rf*: 0.15 (C). IR ν_{max}^{Nujol} cm^{-1}: 3160 (OH), 1615 (CHO). ^{1}H NMR (DMSO-d_6) δ: 10.05 (1H, s, CHO), 6.48 (1H, s, ArH), 4.57 (2H, s, CH_2O), 3.27 (3H, s, OCH_3). Hydroxyl proton signals were hardly observed because of signal broadening.

2,3,4-Triacetoxy-6-methoxymethylbenzaldehyde (21)——Compound **20** (46 mg) was treated with pyridine (1.1 ml) and Ac_2O (0.55 ml) overnight. The solvents were then removed *in vacuo* at room temperature, after which xylene was added and removed similarly. Addition of ether to the residue caused crystallization. Recrystallization from AcOEt–hexane afforded **21** (50 mg, 66%), mp 95—96°C. *Anal.* Calcd for $C_{15}H_{16}O_8$: C, 55.55; H, 4.97. Found: C, 55.73; H, 4.95. *Rf*: 0.22 (A). IR ν_{max}^{Nujol} cm^{-1}: 1770 (OAc), 1690 (CHO). ^{1}H NMR ($CDCl_3$) δ: 10.23 (1H, s, CHO), 7.53 (1H, s, ArH), 4.83 (2H, s, CH_2O), 3.48 (3H, s, OCH_3), 2.37 (3H, s, OAc), 2.30 (6H, s, 2×OAc).

2,3,4-Triacetoxy-6-acetoxymethylbenzaldehyde (22)——A solution of **21** (126 mg) in Ac_2O (6 ml) was cooled to 0°C, then freshly redistilled boron trifluoride etherate (3 ml), which had also been cooled beforehand to 0°C, was added. After 1 h at 0°C, the mixture was poured into ice-cold water and extracted with benzene. The benzene extract was washed with $NaHCO_3$ solution and water, dried over Na_2SO_4, and concentrated. Crystallization of the residue from AcOEt–hexane gave **22** (20 mg, 14%), mp 126—127°C. *Anal.* Calcd for $C_{16}H_{16}O_9$: C, 54.55; H, 4.58. Found: C, 54.56; H, 4.50. *Rf*: 0.16 (A). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 1785 (OAc), 1750 (OAc), 1615 (CHO). ^{1}H NMR ($CDCl_3$) δ: 10.18 (1H, s, CHO), 7.35 (1H, s, ArH), 5.45 (2H, s, CH_2O), 2.35 (3H, s, OAc), 2.30 (6H, s, 2×OAc), 2.17 (3H, s, OAc). MS *m/z* (%): 352 (11, M^+), 310 (10), 292 (21), 268 (71), 250 (15), 226 (55), 208 (11), 166 (100).

3,4,5-Trimethoxyphthalaldehyde (23)——(a) A mixture of **16** (20 g) and SeO_2 (8.8 g) in dioxane (480 ml)

was refluxed fo. 3 h. The mixture was filtered to remove selenium, and the filtrate was concentrated under reduced pressure. The residue was triturated with benzene (200 ml) at room temperature and separated into a crystalline solid and a benzene-soluble fraction. Recrystallization of the solid from benzene gave **24** (1.74 g, 9%), mp 138°C. *Anal.* Calcd for $C_{11}H_{12}O_6$: C, 55.00; H, 5.04. Found: C, 54.73; H, 4.97. *Rf*: 0.15 (A). IR ν_{max}^{Nujol} cm^{-1}: 1740 (C=O). ^1H NMR ($CDCl_3$) δ: 7.03 (1H, s, ArH), 6.64 (1H, d, J=7 Hz, O-CH-O), 4.44 (1H, d, J=7 Hz, OH), 4.05 (3H, s, OCH_3), 3.92 (6H, s, 2×OCH_3), MS m/z (%): 240 (100, M^+), 223 (15), 195 (60). The benzene solution was chromatographed on silica gel (200 g) with benzene–AcOEt (95: 5) as the eluent. The eluate gave **23** (10.26 g, 56%), which formed pale yellow needles from cyclohexane, mp 97—99°C. *Anal.* Calcd for $C_{11}H_{12}O_5$: C, 58.92; H, 5.40. Found: C, 58.84; H, 5.38. *Rf*: 0.27 (A). IR ν_{max}^{Nujol} cm^{-1}: 1690 (CHO), 1675 (CHO). ^1H NMR ($CDCl_3$) δ: 10.50, 10.47 (2×1H, s, CHO), 7.28 (1H, s, ArH), 4.03, 3.98, 3.97 (3×3H, s, OCH_3).

(b) Compound **2** (100 mg), dry acetone (10 ml), anhydrous K_2CO_3 (400 mg), and dimethyl sulfate (0.3 ml) were refluxed under nitrogen for 2 h. After the removal of inorganic solids by filtration, the solution was concentrated under reduced pressure. The residual oil was chromatographed on silica gel (3 g) with $CHCl_3$ as the eluent. Evaporation of the solvent afforded a pale yellow crystalline product **23** (71 mg, 58%) identical with that prepared by method (a).

3,4,5-Trihydroxyphthalaldehyde [Fomecin B] (2)——(a) Using the procedure described for the synthesis of **19**, **23** (396 mg) was demethylated to afford **2** (214 mg, 66%) as yellow needles from AcOEt, mp *ca.* 230°C (dec.). *Anal.* Calcd for $C_8H_6O_5$: C, 52.75; H, 3.32; O, 43.93. Found: C, 52.92; H, 3.47; O, 44.22. *Rf*: 0.30 (C). IR ν_{max}^{KBr} cm^{-1}: 3200 (br, OH), 1690 (sh), 1681 and 1627 (CHO). UV λ_{max}^{EtOH} nm (log ε): 264 (4.32), 287 (3.96), 343 (br, 3.88). ^1H NMR (acetone-d_6) δ: 10.82, 10.07 (2×1H, s, CHO), 7.20 (1H, s, ArH), 12.65, 9.27, 8.87 (3×1H, s, OH). ^{13}C NMR (DMSO-d_6) δc: 194.0, 192.0 (2×CHO), 152.3, 150.8, 137.7, 128.7 (4× aromatic C), 113.6 (2×aromatic C). MS m/z (%): 182 (75, M^+), 154 (48), 153 (100), 125 (19), 79 (10).

(b) Oxidation of **19** (494 mg) was carried out as described for **23**. The product was purified by column chromatography and then by sublimation under high vacuum (0.05 mmHg) at 150°C, giving **2** (98 mg, 27%) as yellow needles, mp *ca.* 230°C (dec.). This product was identical with that prepared by method (a) above.

2,3,4-Trihydroxy-6-hydroxymethylbenzaldehyde [Fomecin A] (1)——A mixture of **2** (264 mg), platinum oxide (62 mg) and EtOH (50 ml) was shaken under a hydrogen atmosphere of 3 atm at room temperature for 3 h. After removal of the catalyst by filtration, the solution was concentrated *in vacuo*. The residue was separated by a simple ten-funnel countercurrent distribution between AcOEt and H_2O. Crude crystalline **1** (131 mg) obtained along with **2** (491 mg) was recrystallized from EtOH–H_2O and gave pure **1** as pale yellow needles (53 mg, 8.4%) which showed no definite melting point but charred gradually above 160°C. *Anal.* Calcd for $C_8H_8O_5$: C, 52.18; H, 4.38. Found: C, 52.20; H, 4.28. *Rf*: 0.23 (D). IR ν_{max}^{KBr} cm^{-1}: 3470, 3140 (OH), 1637 (CHO). UV λ_{max}^{EtOH} nm (log ε): 242 (4.00), 305 (4.12). ^1H NMR (DMSO-d_6) δ: 10.11 (1H, s, CHO), 6.50 (1H, s, ArH), 4.65 (2H, s, CH_2); hydroxyl proton signals were hardly discernible because of signal broadening. ^{13}C NMR (DMSO-d_6) δc: 193.7 (CHO), 152.9, 152.7, 137.9, 130.9, 111.5, 108.0 (6×aromatic C), 60.1 (CH_2). MS m/z (%): 184 (40, M^+), 167 (19), 166 (96), 138 (40), 137 (29), 110 (23), 109 (23), 82 (50), 18 (100). Mp and the UV, IR, NMR, and mass spectra were consistent with those of natural fomecin A, a 81 (22), 53 (25), 39 (25), sample of which was kindly supplied by Dr. Nair.

Fomecin A Tetraacetate (26)——Compound **1** (40 mg) was treated with pyridine (1.0 ml) and Ac_2O (0.5 ml) overnight at room temperature. The solvents were removed *in vacuo* at room temperature, after which xylene was added, and then evaporated off again. Addition of ether to the residue caused crystallization. Recrystallization from AcOEt–hexane afforded **26** (30 mg, 39%), mp 134°C. *Anal.* Calcd for $C_{16}H_{16}O_9$: C, 54.55; H, 4.58. Found: C, 54.58; H, 4.60. *Rf*: 0.15 (A). IR ν_{max}^{Nujol} cm^{-1}: 1779, 1760, 1744 (OAc). UV λ_{max}^{EtOH} nm (log ε): 264 (2.92), 271 (2.90). ^1H NMR ($CDCl_3$) δ: 7.10 (1H, ArH), 2.28 (6H, s, 2×OAc), 2.26, 2.03 (2×3H, s, OAc), ABX-type signals; see the text. MS m/z (%): 293 (2, M^+), 250 (5), 208 (25), 166 (100), 43 (85). This compound was identical with authentic material prepared in a similar way from natural fomecin A on the basis of mixed mp determination and IR and NMR spectra.

3,4,5,6-Tetramethoxyphthalide (25)——(a) A solution of **24** (1.13 g) and *p*-toluenesulfonic acid (15 mg) in MeOH (10 ml) was stirred under reflux for 2 h. The mixture was then evaporated to dryness under reduced pressure. The residue was dissolved in benzene, then the solution was washed with H_2O, dried over Na_2SO_4, and evaporated to dryness. Recrystallization from cyclohexane gave **25** (932 mg, 78%) as colorless needles, mp 86°C. *Anal.* Calcd for $C_{12}H_{14}O_6$: C, 56.69; H, 5.55. Found: C, 56.62; H, 5.53. *Rf*: 0.35 (A). IR ν_{max}^{KBr} cm^{-1}: 1775 (C=O). ^1H NMR ($CDCl_3$) δ: 7.10 (1H, s, ArH), 6.32 (1H, s, CH), 4.00 (3H, s, OCH_3), 3.90 (6H, s, 2×OCH_3), 3.58 (3H, s, OCH_3).

(b) Reaction of **9** (452 mg) with dichloromethyl methyl ether and $TiCl_4$ as described for the formylation of **10** gave **25** (293 mg, 58%) as colorless needles, mp 86°C. This compound was identical with the material prepared by method (a).

Methyl 2-Formyl-3,4,5-trihydroxybenzoate (27)——Demethylation of **24** (240 mg) according to the procedure described for the preparation of **20** afforded **27** (72 mg, 34%) as colorless needles, mp 167—168°C, after recrystallization from H_2O (Norit). *Anal.* Calcd for $C_9H_8O_6 \cdot 1/2H_2O$: C, 48.87; H, 4.10. Found: C, 49.13; H, 4.39. *Rf*: 0.26 (B). IR ν_{max}^{KBr} cm^{-1}: 3660 (broad, OH), 1707 ($COOCH_3$), 1640 (CHO). UV λ_{max}^{EtOH} nm (log ε): 256 (4.04), 318 (3.68). ^1H NMR (acetone-d_6) δ: 10.55 (1H, s, CHO), 7.15 (1H, s, ArH), 3.88 (3H, s,

OCH_3): hydroxyl proton signals were hardly observed because of signal broadening.

2-Formyl-3,4,5-trihydroxybenzoic Acid (28)——Compound **27** (212 mg) was added to 10% H_2SO_4 and the mixture was refluxed for 0.5 h. The precipitate that formed was collected by filtration and recrystallized from H_2O, giving **28** (154 mg, 78%) as colorless needles, mp *ca.* 270°C (dec.). *Anal.* Calcd for $C_8H_6O_6$: C, 48.49; H, 3.05. Found: C, 48.25; H, 3.20. IR ν_{max}^{KBr} cm^{-1}: 3520, 3290 (OH), 1706 (COOH), 1615 (CHO). UV λ_{max}^{EtOH} nm (log ε): 252 (4.19), 307 (3.97). MS *m/z* (%): 198 (45, M^+), 180 (55), 163 (100).

2,3,4-Trihydroxy-5-hydroxymethylbenzaldehyde (30)——A solution of **29** (154 mg) in 4% NaOH (2 ml) was treated with 37% formalin (0.3 ml) and the mixture was stirred for 3 h at room temperature. The mixture was acidified with dilute HCl and extracted with AcOEt. Evaporation of the solvent produced a yellow solid, which upon crystallization from AcOEt gave **30** (62 mg, 34%) as pale yellow needles, mp 132°C (dec.). *Anal.* Calcd for $C_8H_8O_5$: C, 52.18; H, 4.38. Found: C, 51.94; H, 4.32. *Rf*: 0.20 (C). IR ν_{max}^{Nujol} cm^{-1}: 3300 (OH), 1645 (CHO). ^1H NMR (acetone-d_6) δ: 9.85 (1H, s, CHO), 7.33 (1H, s, ArH), 4.73 (2H, s, CH_2); hydroxyl proton signals were not clear because of signal broadening.

3,4-Dihydroxy-5-methoxyphthalaldehyde (31)——Compound *2* (318 mg) was dissolved in 5% aqueous sodium tetraborate solution (16 ml). Dimethyl sulfate (1.0 ml) and a solution of NaOH (0.4 g in 1.6 ml of H_2O) were each added dropwise to the solution at room temperature over a period of 3 h. The solution was left to stand overnight, then acidified with dilute H_2SO_4 and extracted with AcOEt. The residue after evaporation of the solvent was chromatographed on silica gel (15 g) with $CHCl_3$–MeOH (9: 1) as the eluent. The eluate gave **31** (160 mg, 47%), which formed pale yellow needles from AcOEt–benzene, mp 153—154°C (lit.[3] mp 154—155°C). *Anal.* Calcd for $C_9H_8O_5$: C, 55.10; H, 4.11. Found: C, 54.88; H, 4.07. *Rf*: 0.38 (B). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3520, 3500—2400 (broad, OH), 1695, 1640 (CHO). ^1H NMR ($CDCl_3$) δ: 12.60 (1H, s, chelated OH), 10.90, 10.07 (2×1H, s, CHO), 7.07 (1H, s, ArH), 6.13 (1H, s, OH), 4.07 (3H, s, OCH_3).

2,3-Dihydroxy-6-hydroxymethyl-4-methoxybenzaldehyde (33)——Compound **31** (1.0 g) in 99% EtOH was hydrogenated in the presence of 10% Pd-carbon for 10 min at room temperature. After removal of the catalyst by filtration, the solution was concentrated under reduced pressure and gave a crystalline mass. Recrystallization from DMSO–H_2O gave **33** (325 mg, 32%) as pale yellow needles, which did not melt but charred gradually above 160°C. *Anal.* Calcd for $C_9H_{10}O_5$: C, 54.54; H, 5.09. Found: C, 54.32; H, 4.93. IR ν_{max}^{KBr} cm^{-1}: 3490, 3600—2000 (broad, OH), 1632 (CHO). UV λ_{max}^{EtOH} nm (log ε): 240 (3.79), 300 (3.85). ^1H NMR (DMSO-d_6) δ: 10.20 (1H, s, CHO), 6.75 (1H, s, ArH), 4.74 (2H, s, CH_2), 3.88 (3H, s, OCH_3); hydroxyl proton signals were hardly observed because of signal broadening. MS *m/z* (%): 198 (100, M^+), 180 (45), 152 (50).

3,4,5-Triacetoxy-6-methoxy-1*H*,3*H*-benzo[*b*]furan (34)——Acetylation of **33** was carried out as described for that of **1**. Recrystallization of the product from benzene–hexane gave **34** (75 mg, 46%), mp 117—118°C. *Anal.* Calcd for $C_{15}H_{16}O_8$: C, 55.55; H, 4.97. Found: C, 55.90; H, 4.98. *Rf*: 0.19 (A). IR ν_{max}^{Nujol} cm^{-1}: 1775, 1765, 1747 (OAc). UV λ_{max}^{EtOH} nm (log ε): 276 (3.44). ^1H NMR ($CDCl_3$) δ: ABX-like signals; 5.31 (1H, dd, J=13, 1.5 Hz, A), 5.08 (1H, dd, J=13, 0.5 Hz, B), 7.33 (1H, dd, J=1.5, 1.0 Hz, O–CH–O, X); the AB signals were further split by benzylic couplings to ArH; 6.80 (1H, dd, ArH), 3.85 (3H, s, OCH_3), 2.30, 2.25, 2.03 (3×3H, s, OAc).

4-Farnesyloxy-3-hydroxy-5-methoxyphthalaldehyde [Asperugin] (3)——A mixture of **31** (79 mg), *t,t*-farnesylbromide (191 mg),[18] K_2CO_3 (110 mg), and NaI (60 mg) in dry acetone (4 ml) was stirred under reflux for 3 h. The mixture was filtered and the filtrate was concentrated, giving an oil. Thin-layer chromatographic separation (benzene–AcOEt, 9: 1) of the crude product gave **3** (25 mg, 16%) as a viscous oil. *Rf*: 0.47 (A). IR ν_{max}^{film} cm^{-1}: 3500—3300 (br, OH), 1690, 1635 (CHO). ^1H NMR ($CDCl_3$) δ: 12.42 (1H, s, chelated OH), 10.82, 10.08 (2×1H, s, CHO), 7.02 (1H, s, ArH), 5.55 (1H, =CH), 5.12 (2×1H, =CH), 4.74 (2H, d, J=7 Hz, OCH_2), 4.03 (3H, s, OCH_3), 2.03 (4×2H, CH_2), 1.70, 1.62 (4×3H, C–CH_3). MS *m/z* (%): 401 (6, M+1), 205 (44), 197 (100), 137 (34), 81 (61), 69 (90), 31 (37).

Compound **31** was similarly allowed to react with prenyl bromide or geranyl chloride[19] and the products were separated by preparative thin-layer chromatography, giving **32a—d**.

Alkenyloxybenzaldehydes (36)——Methylation of **29** (77 mg) was carried out as described for that of **2**. Recrystallization of the product from H_2O gave **35** (35 mg, 42%), mp 118°C (lit.[20] mp 117—118°C). *Rf*: 0.49 (A). IR ν_{max}^{Nujol} cm^{-1}: 3380 (OH), 1650 (CHO). ^1H NMR ($CDCl_3$) δ: 11.10 (1H, s, chelated OH), 9.75 (1H, s, CHO), 7.13 (1H, d, J=8 Hz, ArH), 6.60 (1H, d, J=8 Hz, ArH), 5.53 (1H, s, OH), 3.98 (3H, s, OCH_3).

Reaction of **35** with allyl bromide, prenyl bromide, geranyl chloride, or farnesyl bromide as described for the synthesis of **3** afforded **36a—l** after thin-layer chromatographic separation.

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