STUDIES ON MACROCYCLIC LACTONE ANTIBIOTICS

XIII†. ANTI-TUBULIN ACTIVITY AND CYTOTOXICITY OF RHIZOXIN DERIVATIVES: SYNTHESIS OF A PHOTOAFFINITY DERIVATIVE

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Chemical modification of the side chain in rhizoxin, a potent antimitotic agent, was attempted in order to study structure-activity relationships and also to devise a probe for photoaffinity labeling of tubulin. An $OsO_4/NaIO_4$ oxidation gave a nor-rhizoxin 20-al (**5**) which was converted to 20-ol (**6**) by a $NaBH_3CN$ reduction. Starting from these two compounds as key intermediates, a series of Wittig reaction products **7**~**12**, and of 20-*O*-acylates **13**~**21** were prepared and their anti-tubulin activity and cytotoxicity were determined. An aryl azide derivative **23** was synthesized as a photoaffinity analogue.

Rhizoxin is an antitumor antibiotic produced by a fungus *Rhizopus chinensis*[1]. It exhibits potent antimitotic activity, through binding to β-tubulin, against most eucaryotic cells including those of animals, plants and fungi[1~4]. We have previously shown that rhizoxin and maytansine share the same binding site on porcine and bovine brain tubulin as well as on fungal tubulin, and that the rhizoxin/maytansine site is different from those for colchicine and for vinblastine[3~5]. Knowledge of the structure-activity relationships of rhizoxin derivatives and of the location and properties of the newly identified rhizoxin/maytansine binding site is essential for understanding the interaction between rhizoxin and tubulin. Such knowledge should facilitate elucidation of interaction between tubulin and other ligands which bind to this site, and also to devise new classes of antimitotic agents.

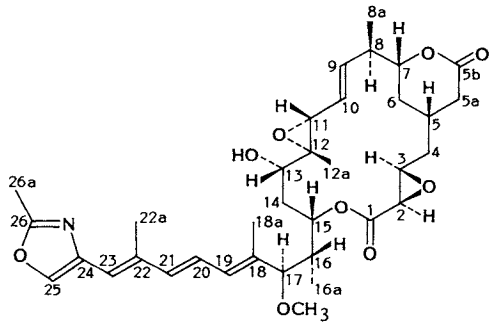
Photoaffinity labeling has been used extensively in the selective labeling of receptor sites in a large variety of biological systems, especially with aryl azide as the photoreactive moiety[6,7]. We have, therefore, undertaken chemical modification of rhizoxin to clarify the modifiable structural elements without loss of anti-tubulin and antimitotic activity.

Modification of The Side Chain Structure

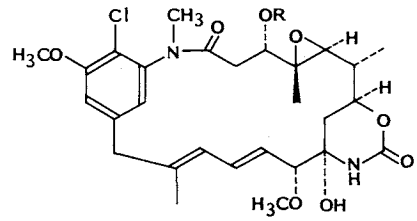
It has already been shown, in the anti-tubulin activity of rhizoxin homologues, that replacement of epoxides to double bond at the 2,3- and 11,12-positions retained activity, whereas hydrolytic opening of these epoxides leading to conformational changes of the 16-membered ring reduced activity. A reductive opening of the 6-membered lactone ring to yield a 5b,7,13-triol derivative or acylation of 13-ol in **1** also caused loss of binding affinity[8]. These facts suggested that the conformation of the 16-membered ring,

† For part XII of the series "Studies on macrocyclic lactone antibiotics", see ref 3.

Fig. 1. Structures of rhizoxin and maytansinoid compounds.

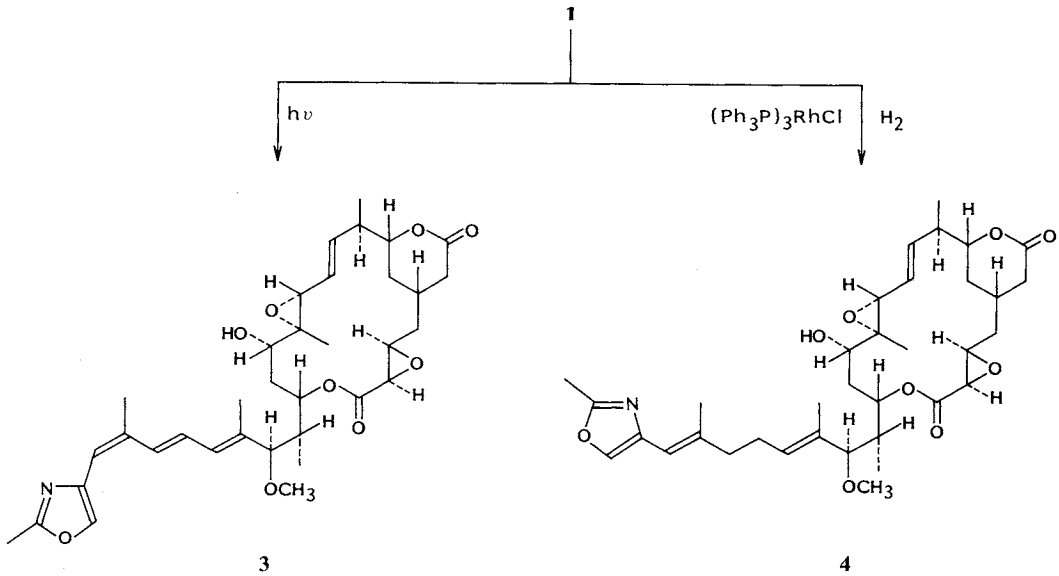


Rhizoxin (**1**)



Maytansine (**2a**) R = $COCHCH_3NCH_3COCH_3$
Ansamitocin P-3 (**2b**) R = $COCH(CH)_3$

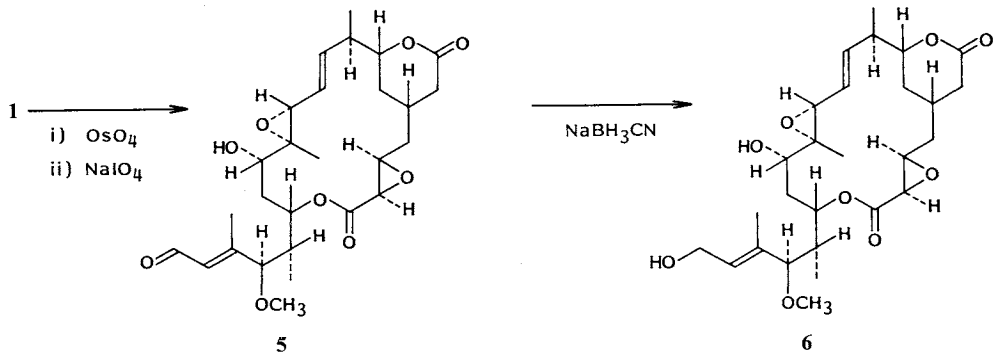
Scheme 1. Isomerization and partial hydrogenation of rhizoxin side chain.



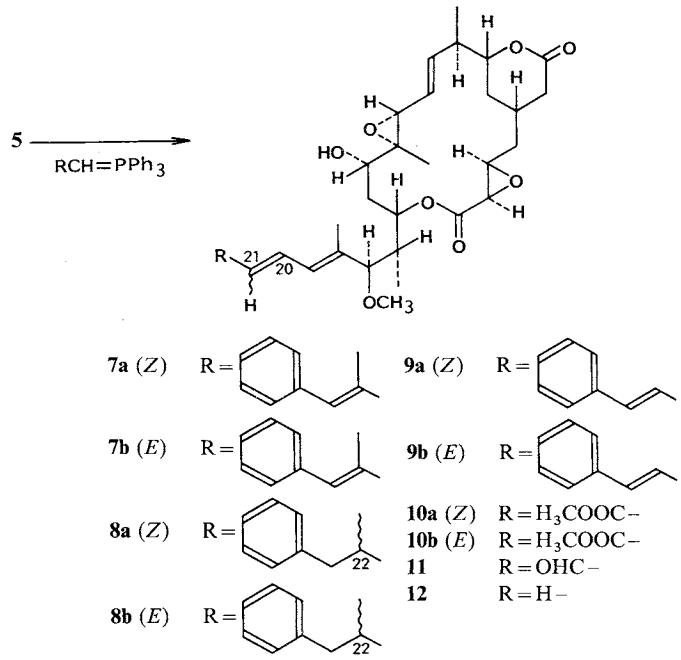
and the partial structures including the 6-membered lactone ring and the hydroxy group at C-13 should be important in binding to tubulin. Whereas rhizoxin and maytansine are structurally similar in that they both contain macrocycles and six-membered acyl rings, maytansine has no such structure as the rhizoxin side chain. Since these two drugs competitively bind to tubulin and were accordingly expected to interact with the receptor site by the same sort of structural elements, it was assumed that the side chain in the rhizoxin molecule should not be essential for binding. Based on this argument, modification of the rhizoxin side chain was attempted.

(22*Z*)-Rhizoxin (**3**) was isolated from the broth of a large scale culture of *R. chinensis* Rh-2 strain and was identified as a photoisomerization product obtained by irradiation of rhizoxin (**1**) by a UV-Auto-Fade Meter. The (22*Z*)-configuration in compound **3** was assigned on the basis of NOE enhancements of the 20-H and 23-H signals, observed by irradiation of 22a-H_3 signal. The compound was, however, formed also by exposure of rhizoxin to room light in low yield and was, therefore, assumed to

Scheme 2. Oxidative cleavage of rhizoxin side chain.



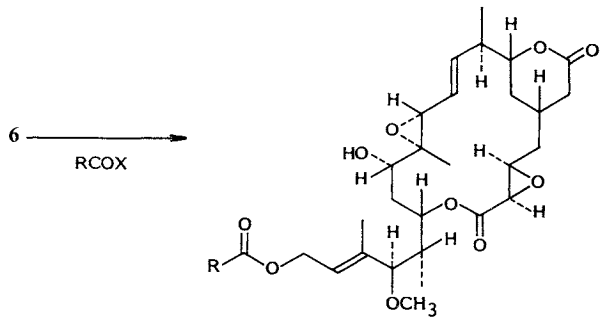
Scheme 3. Witting reaction of aldehyde **5**.

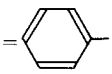


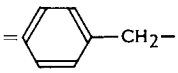
be an artifact. Partial hydrogenation of **1** in benzene over chloro-tris(triphenylphosphine)rhodium catalyst gave 20,21-dihydrorhizoxin (**4**) (Scheme 1). Oxidation of **1** with OsO_4, followed by $NaIO_4$ treatment gave aldehyde **5** which was converted to alcohol **6** with $NaBH_3CN$ in acidic methanol (Scheme 2). A variety of derivatives were prepared from these two key compounds.

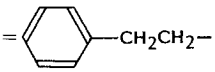
Reactions of aldehyde **5** with a variety of phosphonium ylides $Ph_3P=CHR$ ($R=C(CH_3)=CHPh$, $CH(CH_3)CH_2Ph$, $CH=CHPh$, $COOCH_3$, CHO, H), gave mixtures of respective 20*Z* and 20*E* isomers **7**~**10**, (20*E*)-**11** and **12** (Scheme 3). Purification of the products was generally carried out by thin layer chromatography. The mixtures of **8a** (20*Z*) and **8b** (20*E*), each of which was 1:1 mixture of isomers diastereomeric at C-22, and of **10a** (20*Z*) and **10b** (20*E*) could not however be separated and were subjected to biological tests as mixtures. The **10a**/**10b** ratio was determined to be 3:7 by means of ^1H NMR spectroscopy (see Experimental part) but estimation of the **8a**/**8b** ratio was not possible because of the

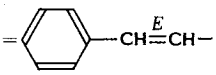
Scheme 4. 20-*O*-Acylation of alcohol **6**.



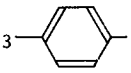
13 R= 

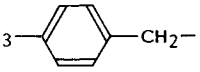
14 R= 

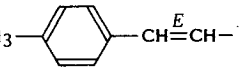
15 R= 

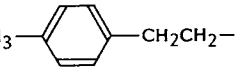
16 R= 

17 R=$CH_3(CH_2)_6$

18 R=N_3 

19 R=N_3 

20 R=N_3 

21 R=N_3 

complexity of the mixture. Products **10** and **11**, prepared with stable ylides were obtained in fairly good yields (quantitative and 62%, respectively). The yields of the others however were less than 60%. Lower yields of the latter products were presumably due to instability of the aldehyde **5** to the basic condition required for ylide formation.

Nor-rhizoxin 20-*O*-acylates **13**~**21** were prepared from alcohol **6** in good yields, either by reaction of **6** with the corresponding acyl chlorides in pyridine, or by treatment of **6** with the corresponding acids in acetonitrile in the presence of dimethylaminopyridine (DMAP) and 1-fluoro-2,4,6-trinitrobenzene (FTNB)[9] as catalysts (Scheme 4). The derivatives with the *p*-azidophenyl group **18**~**21** were prepared by the coupling of **6** with the respective azidoacids, derived by reaction of the corresponding *p*-aminophenyl acids with $NaNO_2$ in acidic media, followed by treatment with NaN_3[10,11].

Anti-tubulin Activity and Cytotoxicity

Inhibition of tubulin assembly and *in vitro* cytotoxicity against P388 leukemia cells were assayed for rhizoxin (**1**) and its derivatives **3**~**21**. The results are shown in Table 1. The structural effect on tubulin assembly can be summarized as follows: 1) Isomerization of the C-22=C-23 double bond from *E* to *Z* and hydrogenation of the C-20=C-21 double bond did not change the activity. 2) Nor-rhizoxin 20-al (**5**) and 20-ol (**6**) showed little activity. 3) The Wittig reaction products (**7**~**12**) derived from **5** generally showed activity comparable to rhizoxin, and 20*E*-isomers seemed to have superior potency to the corresponding 20*Z*-isomers. 4) In most cases, 20-*O*-acylation of **6** led to recovery of potency, but they were still much less active than the carbon chain derivatives, **3**, **4**, and **7**~**12**.

It should be noted that a minimum structural change from O=C-20 in **5** to CH_2=C-20 in **12** conferred much higher activity (more than 25-fold). This might suggest a possible negative effect on binding to tubulin caused by the oxygen function at this position. Nevertheless, we could demonstrate that the side

Table 1. Cytotoxicity against leukemia P388 and inhibitory activity against tubulin assembly of rhizoxin derivatives.

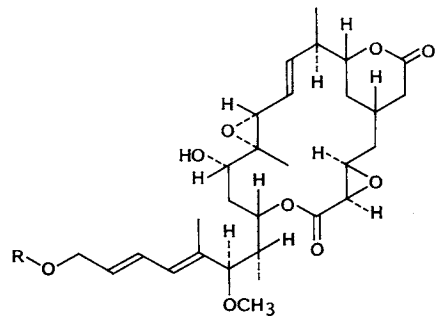
Compound	IC_{50} (μM)		Compound	IC_{50} (μM)	
	P388	Tubulin		P388	Tubulin
Rhizoxin (**1**)	0.00043	3.4	**12**	0.063	3.8
3	0.002	3.7	**13**	12.42	35.0
4	0.03	3.6	**14**	4.15	30.0
5	2.87	>100.0	**15**	14.1	25.0
6	23.62	>100.0	**16**	1.8	20.0
7a	0.016	5.3	**17**	10.25	>100.0
7b	0.0012	3.6	**18**	2.9	17.0
8a+8b	0.17	7.4	**19**	12.28	20.0
9a	0.0036	8.0	**20**	1.84	14.0
9b	0.0005	3.8	**21**	1.98	15.0
10a+10b	0.71	6.8	**22**	2.53	9.0
11	0.0085	7.6	**23**[a]	—	4.5[a]

[a] The IC_{50} value was measured separately from the others and was comparable to that for **1** measured at the same time.

chain in rhizoxin is modifiable without loss of binding affinity to tubulin.

Cytotoxicity of these compounds should essentially be due to their anti-tubulin activity, but the observed values of these two activities were not always parallel. The result apparently indicates that the side chain moiety should be playing some role in other functions, such as cell permeability.

Fig. 2. Structures of compounds **22** and **23**.



22 R=H

23 R=N_3—C$_6$H$_4$—CO—

Preparation of A Photoaffinity Ligand

The aldehyde **11** prepared by a Wittig reaction was treated with $NaBH_3CN$ in acidic methanol to give the corresponding alcohol **22** which was converted to the *p*-azidobenzoate **23** (Fig. 2) by coupling with *p*-azidobenzoic acid in the presence of FTNB[9]. The azidobenzoate **23** showed 50% and 100% inhibition of microtubule assembly at 4.5 μM and 10 μM, respectively. The observed inhibitory activity was as high as that of rhizoxin under control condition. Radioactive **23** should be preparable with [1-^{14}C]*p*-azidobenzoic acid derived from commercially available [1-^{14}C]*p*-aminobenzoic acid, and should facilitate photoaffinity labeling of rhizoxin/maytansine site.

Experimental

General

The Wittig reaction products, unless stated were homogeneous by TLC, and 20-*O*-acylation products were homogeneous by both TLC and HPLC. UV spectra were measured on a Shimadzu UV-300 Photospectrometer, the maxima are given in nm (extinction ε). IR spectra were measured on a Japan Spectroscopic Apparatus (model A-102) and recorded in cm^{-1}. Mass spectra were measured on Jeol JMS-HX110 and recorded in *m/z*. ^{1}H NMR were measured on Jeol GX-400 (400 MHz) and GX-500

(500 MHz) instruments; chemical shifts are given in ppm (δ) relative to TMS as internal standard; s = singlet, d = doublet, dd = doublet of doublets, t = triplet, dt = doublet triplet, m = multiplet, br = broad, coupling constants J were given in Hz. HPLC was performed on a Shimadzu apparatus (model LC-7A), the ODS column was normally used.

Purification of Microtubules Protein and Polymerization Assay

Microtubule protein was prepared from porcine brain tubulin as described in a previous paper[8]. Polymerization of tubulin was followed by turbidity measurement at 37 °C in MES buffer solution containing 2 mg of protein per ml of solution. The turbidity was measured at 400 nm on Shimadzu UV-300 Photospectrometer. The compounds tested were added as a DMSO solution.

Evaluation of Cytotoxicity

P388 cells were suspended in RPMI-1640 medium containing 5% FCS, 5 μM HEDS, 100 U/ml penicillin and 100 μg streptomycin. Graded concentrations of drug were added as a DMSO solution immediately after seeding the cells. After 48 hours of continuous drug exposure, the tumor cells were counted by a Coulter Counter (model ZM). IC_{50} was determined by comparison with control culture.

Preparation of (22Z)-Rhizoxin (**3**)

Rhizoxin (**1**) (1 g) in acetonitrile (5 ml) was irradiated with a UV-Auto-Fade Meter for 150 minutes. The reaction mixture was separated by repeated HPLC using ODS column eluted with acetonitrile to yield **3** (60 mg): $C_{35}H_{47}NO_9$; EI-MS 625 (M^+); UV (EtOH) 311 (35,600); ^1H NMR ($CDCl_3$) 0.82 (1H, dt, 4-H), 0.95 (1H, dt, 6-H), 1.02 (3H, d, 16a-H), 1.19 (3H, d, 8a-H), 1.42 (3H, s, 12a-H), 1.83 (3H, br s, 18a-H), 1.89~1.96 (2H, m, 6-H, 14-H), 2.02 (3H, s, 22a-H), 2.02~2.13 (3H, m, 5a-H, 5-H, 14-H), 2.27~2.45 (3H, m, 8-H, 4-H, 16-H), 2.48 (3H, s, 26a-H), 2.74 (1H, dd, 5a-H), 2.97 (1H, d, 2-H), 3.03 (1H, br d, 13-H), 3.13 (1H, d, 11-H), 3.17 (3H, s, OCH_3), 3.25 (1H, dt, 3-H), 3.30 (1H, d, 17-H), 3.87 (1H, m, 7-H), 4.56 (1H, dd, 15-H), 5.36 (1H, dd, 10-H), 5.61 (1H, dd, 9-H), 6.06 (1H, s, 23-H), 6.20 (1H, d, $J_{19,20}=12.0$, 19-H), 6.58 (1H, dd, $J_{19,20}=12.0$, $J_{20,21}=16.0$, 20-H), 7.45 (1H, s, 25-H), 7.50 (1H, d, $J_{20,21}=16.0$, 21-H).

The geometry of the C-22=C-23 double bond was assigned on the basis of NOE spectroscopy. Irradiation at 2.02 (22a-H) gave rise to enhancements of 20-H and 23-H signals appearing at δ 6.58 and 6.06, respectively.

Preparation of 20,21-Dihydrorhizoxin (**4**)

To rhizoxin (300 mg) and chloro-tris(triphenylphosphine)rhodium (9 mg) in a sealed flask was added degassed benzene (2 ml). The whole was kept stirred under a hydrogen atmosphere for 24 hours at 60~65°C. TLC separation (benzene - acetone, 3 : 1) of the reaction mixture gave **4** (20 mg): $C_{35}H_{49}NO_9$; EI-MS 627 (M^+); UV (EtOH) 232 (10,000); ^1H NMR ($CDCl_3$) 0.70~2.40 (15H, m), 0.95 (3H, s, 16a-H), 1.21 (3H, d, 8a-H), 1.39 (3H, s, 12a-H), 1.63 (3H, s, 18a-H), 1.86 (3H, s, 22a-H), 2.44 (3H, s, 26a-H), 2.65~3.30 (6H, m), 3.08 (3H, s, OCH_3), 3.89 (1H, m, 7-H), 4.57 (1H, m, 15-H), 5.35 (2H, m, 10-H, 19-H), 5.62 (1H, dd, 9-H), 5.90 (1H, s, 23-H), 7.40 (1H, s, 25-H).

Preparation of Nor-rhizoxin-20-al (**5**)

To rhizoxin (**1**) (100 mg; 0.16 mmol) in pyridine was added OsO_4 (45 mg; 0.18 mmol) under ice cooling. The reaction temperature was then gradually raised up to room temperature, and the mixture was stirred for another 30 minutes at room temperature. To the reaction mixture $NaHSO_3$ (120 mg; 1.1 mmol) in water (2 ml) was added. The whole was kept stirred for 1 hour, and the solution was concentrated *in vacuo* to give a residual mixture which was dissolved in EtOAc. The solution was washed with water and dried over sodium sulfate. After evaporation of the solvent, the residue was dissolved in dioxane and treated with aq $NaIO_4$ (10 mg; 0.08 mmol in 2 ml water) for 12 hours at room temperature. The residue after evaporation of the solvent was dissolved in EtOAc, and organic layer was washed with water, dried over sodium sulfate, and evaporated *in vacuo* to give a crude product. Silica gel chromatography of the crude product eluted with benzene - EtOAc (3 : 2) gave compound **5** (66 mg) in 82% yield: $C_{27}H_{38}O_9$; FAB-MS 507 $(M+H)^+$; IR (KBr) 3460, 1740, 1675; ^1H NMR ($CDCl_3$) 0.8~2.4 (10H, m), 0.97 (3H, d, 16a-H),

1.25 (3H, d, 8a-H), 1.42 (3H, s, 12a-H), 2.15 (3H, s, 18a-H), 2.7~3.4 (6H, m), 3.21 (3H, s, OCH_3), 3.90 (1H, m, 7-H), 4.65 (1H, dd, 15-H), 5.38 (1H, dd, 10-H), 5.65 (1H, dd, 9-H), 5.98 (1H, d, $J_{19,20}$=9.4, 19-H), 10.1 (1H, d, $J_{19,20}$=9.4, 20-H).

Preparation of Nor-rhizoxin-20-ol (**6**)

To aldehyde **5** (70 mg; 0.14 mmol) in methanol (5 ml) was added $NaBH_3CN$ (14 mg; 0.22 mmol). The reaction solution was kept acidic with dil HCl under control by methyl red (pH 4.2~6.2) and was stirred for 30 minutes at room temperature. After evaporation of the solvent, the residue was dissolved in EtOAc and the organic layer was worked up as usual. Separation by silica gel column chromatography eluted with benzene-EtOAc (1:1) afforded alcohol **6** (40 mg) in 72% yield: $C_{27}H_{40}O_9$; FAB-MS 509 $(M+H)^+$; IR (KBr) 3460, 1740; ^1H NMR ($CDCl_3$) 0.8~2.4 (10H, m), 1.00 (3H, d, 16a-H), 1.23 (3H, d, 8a-H), 1.43 (3H, s, 12a-H), 1.68 (3H, s, 18a-H), 2.7~3.3 (6H, m), 3.17 (3H, s, OCH_3), 3.90 (1H, m, 7-H), 4.24 (2H, dd, $J_{19,20}$=4.0 and 8.0, 20-H), 4.64 (1H, dd, 15-H), 5.38 (1H, dd, 10-H), 5.47 (1H, dd, $J_{19,20}$=4.0 and 8.0, 19-H), 5.66 (1H, 9-H).

Preparations of Wittig Reaction Products (**7**~**12**) from Aldehyde **5**

Compound **7a** (20*Z*) and **7b** (20*E*)

To a suspension of *trans*-$PhCH{=}C(CH_3)CH_2PPh_3Br$ (200 mg; 0.42 mmol) in abs THF (1 ml) was dropwise added 50 μl (0.08 mmol) of *n*-BuLi (1.6 M hexane solution) under nitrogen atmosphere and the whole was stirred for 10 minutes at room temperature to yield a ylide solution. The ylide solution (0.25 ml) was added to the aldehyde **5** (10 mg; 0.02 mmol) dissolved in 1 ml of abs THF under nitrogen atmosphere at −78°C. The reaction temperature was raised up to −30°C. To the solution satd NH_4Cl (1 ml) was added and stirred for another 5 minutes. After evaporation of the solvent, the residue was extracted with EtOAc. The extract was worked up as usual. Preparative TLC of the crude mixture developed with benzene-acetone (5:2) gave 3.0 mg and 0.8 mg of **7a** (20*Z*) and **7b** (20*E*) derivatives, respectively.

7a (20*Z*): $C_{37}H_{48}O_8$; FAB-MS 621 $(M+H)^+$; UV (EtOH) 310 (33,800), 321 (38,800), 337 (27,200); IR (KBr) 3460, 1740; ^1H NMR ($CDCl_3$) 0.75~2.10 (8H, m), 0.99 (3H, s, 16a-H), 1.23 (3H, d, 8a-H), 1.38 (3H, s, 12a-H), 1.81 (3H, s, 18a-H), 2.09 (3H, s, 22a-H), 2.25~3.30 (9H, m), 3.15 (3H, s, OCH_3), 3.88 (1H, m, 7-H), 4.60 (1H, dd, 15-H), 5.35 (1H, dd, 10-H), 5.64 (1H, dd, 9-H), 6.05 (1H, d, $J_{19,20}$=12.6, 19-H), 6.28 (1H, dd, $J_{19,20}$=12.6, $J_{20,21}$=10.8, 20-H), 6.49 (1H, s, 23-H), 6.55 (1H, d, $J_{20,21}$=12.6, 21-H), 7.20~7.37 (5H, m, Ph).

7b (20*E*): $C_{37}H_{48}O_8$; FAB-MS 621 $(M+H)^+$; UV (EtOH) 310 (43,500), 323 (53, 200), 340 (41, 000); IR (KBr) 3460, 1740; ^1H NMR ($CDCl_3$) 0.75~2.10 (8H, m), 1.00 (3H, d, 16a-H), 1.22 (3H, d, 8a-H), 1.38 (3H, s, 12a-H), 1.84 (3H, s, 18a-H), 2.05 (3H, s, 22a-H), 2.20~3.30 (9H, m), 3.15 (3H, s, OCH_3), 3.88 (1H, m, 7-H), 4.63 (1H, m, 15-H), 5.35 (1H, dd, 10-H), 5.63 (1H, dd, 9-H), 6.07 (1H, d, $J_{19,20}$=12.5, 19-H), 6.42 (1H, d, $J_{20,21}$=16.3, 21-H), 6.55 (1H, s, 23-H), 6.56 (1H, dd, $J_{19,20}$=12.5, $J_{20,21}$=16.3, 20-H), 7.20~7.37 (5H, m, Ph).

Compounds **8a** (20*Z*) and **8b** (20*E*)

dl-$PhCH_2(CH_3)CH_2PPh_3Br$ was likewise reacted with 10 mg of aldehyde **5**. Preparative TLC gave a mixture (1.8 mg) of **8a** and **8b** both of which were respective diastereomeric mixture: $C_{37}H_{50}O_8$; FAB-MS 623 $(M+H)^+$; IR (KBr) 3450, 1740; ^1H NMR ($CDCl_3$) 0.70~3.70 (38H, m), 4.40~4.60 (1H, m), 5.20~6.25 (5H, m), 7.00~7.50 (5H, m, Ph).

Compound **9a** (20*Z*) and **9b** (20*E*)

$PhCH{=}CHCH_2PPh_3Br$ was likewise reacted with 10 mg of **5** at 10°C. Preparative TLC of the crude mixture developed with benzene-acetone (3:1) gave a mixture of **9a** (20*Z*) and **9b** (20*E*) (6.9 mg). The mixture was further separated by TLC developed with EtOAc-CH_2Cl_2 (1:3) to give **9a** (4.5 mg) and **9b** (2.4 mg).

9a (20*Z*): $C_{36}H_{46}O_8$; FAB-MS 607 $(M+H)^+$; UV (EtOH) 305 (25,000); IR (KBr) 3460, 1740; ^1H NMR ($CDCl_3$) 0.75~2.15 (8H, m), 1.02 (3H, d, 16a-H), 1.20 (3H, d, 8a-H), 1.37 (3H, s, 12a-H), 1.80 (3H, d, 18a-H), 2.25~2.35 (9H, m), 3.18 (3H, s, OCH_3), 3.85 (1H, m, 7-H), 4.57 (1H, m, 15-H), 5.32 (1H,

dd, 10-H), 5.59 (1H, dd, 9-H), 6.20 (1H, dd, $J_{20,21}=11.1$ and $J_{21,22}=11.1$, 21-H), 6.28 (1H, dd, $J_{19,20}=11.1$, $J_{20,21}=11.1$, 20-H), 6.54 (1H, d, $J_{19,20}=11.1$, 19-H), 6.58 (1H, d, $J_{22,23}=16.8$, 23-H), 6.85 (1H, dd, $J_{21,22}=11.1$, $J_{22,23}=16.8$, 22-H), 7.20~7.50 (5H, m).

9b (20*E*): $C_{36}H_{46}O_8$; FAB-MS 607 $(M+H)^+$; UV (EtOH) 308 (34,700); IR (KBr) 3460, 1740; ^1H NMR ($CDCl_3$) 0.70~2.15 (8H, m), 1.00 (3H, d, 16a-H), 1.20 (3H, d, 8a-H), 1.41 (3H, s, 12a-H), 1.81 (3H, s, 18a-H), 2.20~3.30 (9H, m), 3.15 (3H, s, OCH_3), 3.88 (1H, m, 7-H), 4.57 (1H, m, 15-H), 5.35 (1H, dd, 10-H), 5.61 (1H, dd, 9-H), 6.05 (1H, d, $J_{19,20}=11.0$, 19-H), 6.38 (1H, dd, $J_{20,21}=14.8$, $J_{21,22}=11.0$, 21-H), 6.57 (1H, d, $J_{22,23}=15.8$, 23-H), 6.58 (1H, dd, $J_{19,20}=11.0$, $J_{20,21}=14.8$, 20-H), 6.85 (1H, dd, $J_{21,22}=11.0$, $J_{22,23}=15.8$, 22-H), 7.20~7.45 (5H, m, Ph).

Compound **10a** (20*Z*) and **10b** (20*E*)

To a solution of aldehyde **5** (10 mg, 0.02 mmol) in dichloroethane (0.3 ml) was added $CH_3OCOCHPPh_3$ (13 mg; 0.03 mmol). The whole was heated to 50°C for 2 hours. After evaporation of the solvent, the residual mixture was separated by TLC developed with benzene-acetone (5:2) to give a mixture (11 mg) of **10a** (20*Z*) and **10b** (20*E*) in 3:7 ratio: $C_{30}H_{42}O_{10}$; FAB-MS 563 $(M+H)^+$; IR (KBr) 3470, 1740, 1725; ^1H NMR ($CDCl_3$) 0.70~2.50 (11H, m), 0.95 (3H, d, 16a-H), 1.21 (3H, d, 8a-H), 1.37 (3H, s, 12a-H), 1.85 (0.9H, s, 18a-H of **10a**), 1.88 (2.1H, s, 18a-H of **10b**), 2.60~3.40 (6H, m), 3.14 (0.9H, s, OCH_3 of **10a**), 3.15 (2.1H, s, OCH_3 of **10a**), 3.70 (0.9H, s, $COOCH_3$ of **10b**), 3.77 (2.1H, s, $COOCH_3$ of **10b**), 3.88 (1H, m, 7-H), 4.52 (1H, m, 15-H), 5.35 (1H, dd, 10-H), 5.57 (0.3H, d, 19-H of **10a**), 5.65 (1H, dd, 9-H), 5.72 (0.3H, d, $J_{20,21}=12.5$, 21-H of **10a**), 5.87 (0.7H, d, $J_{20,21}=17.0$, 21-H of **10b**), 6.08 (0.7H, d, 19-H of **10b**), 6.90 (0.3H, t, 20-H of **10a**), 7.59 (0.7H, dd, 20-H of **10b**).

Compound **11** (20*E*)

To aldehyde **5** (30 mg; 0.059 mmol) in benzene (0.3 ml) was added $CHOCHPPh_3$ (22 mg; 0.072 mmol). The reaction mixture was stirred for 72 hours at 37°C. After evaporation of the solvent, the residual mixture was separated by TLC developed with benzene-acetone (5:2) to give **11** (20*E*) (20 mg) as the sole product: $C_{29}H_{40}O_9$; FAB-MS 533 $(M+H)^+$; UV (EtOH) 282 (24,700); IR (KBr) 3460, 1740, 1680; ^1H NMR ($CDCl_3$) 0.75~2.40 (11H, m), 0.99 (3H, d, 16a-H), 1.22 (3H, d, 8a-H), 1.40 (3H, s, 12a-H), 1.94 (3H, s, 18a-H), 2.65~3.35 (6H, m), 3.18 (3H, s, OCH_3), 3.88 (1H, m, 7-H), 4.58 (1H, dd, 15-H), 5.36 (1H, dd, 10-H), 5.62 (1H, dd, 9-H), 6.16 (1H, dd, $J_{20,21}=15.4$, $J_{21,22}=7.7$, 21-H), 6.25 (1H, d, $J_{19,20}=11.4$, 19-H), 7.41 (1H, dd, $J_{19,20}=11.4$, $J_{20,21}=15.4$, 20-H), 9.61 (1H, d, $J_{21,22}=7.7$, CHO).

Compound **12**

To a suspension of CH_3PPh_3Br (23 mg; 0.064 mmol) in abs THF (0.5 ml) was dropwise added 30 μl (0.048 mmol) of *n*-BuLi (1.6 M hexane solution) under nitrogen atmosphere and the whole was stirred for 15 minutes at room temperature to yield a ylide solution. The ylide solution was added to aldehyde **5** (5.20 mg; 0.039 mmol) in abs THF (0.5 ml) at −78°C. The reaction temperature was raised slowly to 30°C in 1 hour. The reaction was stopped by addition of acetic acid (10 μl). To the reaction mixture water was added and the mixture was extracted with EtOAc. The organic layer was washed with water, dried over sodium sulfate. After evaporation of the solvent the residue was separated by TLC developed with ether-EtOAc (4:1) to give **12** (4 mg): $C_{28}H_{40}O_8$; FAB-MS 505 $(M+H)^+$; UV (EtOH) 230 (21,000); IR (KBr) 3450, 1740; ^1H NMR ($CDCl_3$) 0.70~2.15 (8H, m), 0.99 (3H, d, 16a-H), 1.22 (3H, d, 8a-H), 1.41 (3H, s, 12a-H), 1.76 (3H, s, 18a-H), 2.15~3.30 (9H, m), 3.14 (3H, s, OCH_3), 3.88 (1H, m, 7-H), 4.56 (1H, dd, 15-H), 5.12 (1H, br d, 21-H), 5.20 (1H, br d, 21-H), 5.35 (1H, dd, 10-H), 5.63 (1H, dd, 9-H), 5.95 (1H, d, $J_{19,20}=10.3$, 19-H), 6.60 (1H, dt, 20-H).

Preparation of 20-*O*-Acylates (**13**~**21**) of Alcohol **6**

Benzoate **13**: Alcohol **6** (15 mg; 0.03 mmol) in CH_2Cl_2 (1 ml) was treated with benzoyl chloride (6 mg; 0.045 mmol) in the presence of DMAP (7 mg; 0.059 mmol) for 20 minutes at room temperature. Usual work up and silica gel column chromatography (benzene-EtOAc, 2:1) afforded **13** in 83% yield: $C_{34}H_{44}O_{10}$; FAB-MS 613 $(M+H)^+$; IR (KBr) 3460, 1740, 1720; ^1H NMR ($CDCl_3$) 0.75~2.40 (10H, m), 1.00 (3H, d, 16a-H), 1.24 (3H, d, 8a-H), 1.38 (3H, s, 12a-H), 1.81 (3H, s, 18a-H), 2.70~3.30 (6H, m), 3.18 (3H, s, OCH_3), 3.87 (1H, m, 7-H), 4.61 (1H, dd, 15-H), 4.85 (1H, dd, 20-H), 4.98 (1H, dd, 20-H), 5.33

(1H, dd, 10-H), 5.50 (1H, dd, 9-H), 5.65 (1H, dd, 19-H), 7.44 (2H, d, Ph), 7.55 (1H, t, Ph), 8.02 (2H, d, Ph).

Acyl derivatives **14**~**17** were prepared similarly with corresponding acid chlorides in 70~80% yields.

Phenylacetate **14**: $C_{35}H_{46}O_{10}$; FAB-MS 627 $(M+H)^+$; IR (KBr) 3460, 1740; ^1H NMR ($CDCl_3$) 0.75~2.40 (10H, m), 0.98 (3H, d, 16a-H), 1.24 (3H, d, 8a-H), 1.41 (3H, s, 12a-H), 1.71 (3H, s, 18a-H), 2.70~3.30 (6H, m), 3.11 (3H, s, OCH_3), 3.62 (2H, s, $PhCH_2$), 3.88 (1H, m, 7-H), 4.58 (1H, dd, 15-H), 4.63 (1H, dd, 20-H), 4.74 (1H, dd, 20-H), 5.35 (1H, dd, 10-H), 5.45 (1H, dd, 19-H), 5.58 (1H, dd, 9-H), 7.30 (5H, m, Ph).

Phenylpropionate **15**: $C_{36}H_{48}O_{10}$; FAB-MS 641 $(M+H)^+$; IR (KBr) 3470, 1740; ^1H NMR ($CDCl_3$) 0.75~2.40 (10H, m), 0.98 (3H, d, 16a-H), 1.20 (3H, d, 8a-H), 1.41 (3H, s, 12a-H), 1.72 (3H, s, 18a-H), 2.63 (2H, t, $PhCH_2CH_2$), 2.70~3.35 (6H, m), 2.92 (2H, t, $PhCH_2CH_2$), 3.14 (3H, s, OCH_3), 3.79 (1H, m, 7-H), 4.60 (1H, dd, 15-H), 4.63 (1H, dd, 20-H), 4.70 (1H, dd, 20-H), 5.35 (1H, dd, 10-H), 5.47 (1H, dd, 19-H), 5.58 (1H, dd, 9-H), 7.25 (5H, m, Ph).

Cinnamate **16**: $C_{36}H_{46}O_{10}$; FAB-MS 639 $(M+H)^+$; IR (KBr) 3470, 1740, 1715; ^1H NMR ($CDCl_3$) 0.75~2.40 (10H, m), 1.00 (3H, d, 16a-H), 1.11 (3H, d, 8a-H), 1.40 (3H, s, 12a-H), 1.80 (3H, s, 18a-H), 2.70~3.30 (6H, m), 3.18 (3H, s, OCH_3), 3.87 (1H, m, 7-H), 4.59 (1H, dd, 15-H), 4.72 (1H, dd, 20-H), 4.85 (1H, dd, 20-H), 5.31 (1H, dd, 10-H), 5.42 (1H, dd, 9-H), 5.62 (1H, dd, 19-H), 6.42 (1H, d, $PhCH=CH$), 7.39 (3H, m, Ph), 7.55 (2H, d, Ph), 7.68 (1H, d, $PhCH=CH$).

Octanoate **17**: $C_{35}H_{54}O_{10}$; FAB-MS 635 $(M+H)^+$; IR (KBr) 3460, 1740; ^1H NMR ($CDCl_3$) 0.75~2.40 (20H, m), 0.88 (3H, t, $CH_3(CH_2)_6CO$), 0.98 (3H, d, 16a-H), 1.24 (3H, d, 8a-H), 1.42 (3H, s, 12a-H), 1.72 (3H, s, 18a-H), 2.28 (2H, t, $CH_3(CH_2)_5CH_2CO$), 2.70~3.30 (6H, m), 3.15 (3H, s, OCH_3), 3.90 (1H, m, 7-H), 4.58 (1H, dd, 15-H), 4.62 (1H, dd, 20-H), 4.68 (1H, dd, 20-H), 5.38 (1H, dd, 10-H), 5.48 (1H, dd, 19-H), 5.66 (1H, dd, 9-H).

p-Azidobenzoate **18**: To a solution of alcohol **6** (20 mg; 0.039 mmol) and DMAP (10 mg; 0.083 mmol) in acetonitrile (1 ml) was added a suspension of *p*-azidobenzoic acid (7.9 mg; 0.043 mmol) and FTNB (10 mg; 0.043 mmol) in acetonitrile (1 ml). The mixture was stirred for 30 minutes at room temperature. After evaporation of the solvent, the residual mixture was separated by silica gel column chromatography eluted with benzene-EtOAc (3:1) to give **18** (22 mg): $C_{34}H_{43}N_3O_{10}$; FAB-MS 654 $(M+H)^+$; IR (KBr) 3470, 2140, 1740, 1715; ^1H NMR ($CDCl_3$) 0.75~2.40 (10H, m), 1.00 (3H, d, 16a-H), 1.24 (3H, d, 8a-H), 1.39 (3H, s, 12a-H), 1.80 (3H, s, 18a-H), 2.70~3.30 (6H, m), 3.16 (3H, s, OCH_3), 3.90 (1H, m, 7-H), 4.60 (1H, dd, 15-H), 4.84 (1H, dd, 20-H), 4.97 (1H, dd, 20-H), 5.34 (1H, dd, 10-H), 5.52 (1H, dd, 9-H), 5.52 (1H, dd, 9-H), 5.53 (1H, dd, 19-H), 7.08 (2H, d, Ph), 8.00 (2H, d, Ph).

The esters **19**~**21** were prepared as in the case of compound **18** in 60~75% yields.

p-Azidophenylacetate **19**: $C_{35}H_{45}N_3O_{10}$; FAB-MS 668 $(M+H)^+$; IR (KBr) 3470, 2130, 1735; ^1H NMR ($CDCl_3$) 0.75~2.40 (10H, m), 0.97 (3H, d, 16a-H), 1.24 (3H, d, 8a-H), 1.42 (3H, s, 12a-H), 1.71 (3H, s, 18a-H), 2.70~3.30 (6H, m), 3.13 (3H, s, OCH_3), 3.58 (2H, s, $PhCH_2$), 3.89 (1H, m, 7-H), 4.59 (1H, dd, 15-H), 4.64 (1H, dd, 20-H), 4.74 (1H, dd, 20-H), 5.36 (1H, dd, 10-H), 5.45 (1H, dd, 19-H), 5.57 (1H, dd, 9-H), 6.98 (2H, d, Ph), 7.27 (2H, d, Ph).

p-Azidocinnamate **20**: $C_{36}H_{45}N_3O_{10}$; FAB-MS 680 $(M+H)^+$; IR (KBr) 3450, 2130, 1740, 1710; ^1H NMR ($CDCl_3$) 0.75~2.40 (10H, m), 1.00 (3H, d, 16a-H), 1.15 (3H, d, 8a-H), 1.40 (3H, s, 12a-H), 1.88 (3H, s, 18a-H), 2.70~3.30 (6H, m), 3.16 (3H, s, OCH_3), 3.84 (1H, m, 7-H), 4.58 (1H, dd, 15-H), 4.70 (1H, dd, 20-H), 4.88 (1H, dd, 20-H), 5.34 (1H, dd, 10-H), 5.46 (1H, dd, 9-H), 5.59 (1H, dd, 19-H), 6.35 (1H, d, $PhCH=CH$), 7.03 (2H, d, Ph), 7.55 (2H, d, Ph), 7.65 (1H, d, $PhCH=CH$).

3-(*p*-Azidophenyl)propionate **21**: $C_{36}H_{47}N_3O_{10}$; FAB-MS 682 $(M+H)^+$; IR (KBr) 3470, 2120, 1740; ^1H NMR ($CDCl_3$) 0.75~2.40 (10H, m), 0.99 (3H, d, 16a-H), 1.23 (3H, d, 8a-H), 1.43 (3H, s, 12a-H), 1.71 (3H, s, 18a-H), 2.61 (2H, t, $PhCH_2CH_2$), 2.70~3.30 (6H, m), 2.90 (2H, t, $PhCH_2CH_2$), 3.14 (3H, s, OCH_3), 3.86 (1H, m, 7-H), 4.60 (1H, dd, 15-H), 4.62 (1H, dd, 20-H), 4.69 (1H, dd, 20-H), 5.37 (1H, dd, 10-H), 5.44 (1H, dd, 19-H), 5.60 (1H, dd, 9-H), 6.95 (2H, d, Ph), 7.19 (2H, d, Ph).

Preparation of Alcohol **22** from Aldehyde **11**

To aldehyde **11** (10 mg; 0.02 mmol) in methanol (0.5 ml) was added $NaBH_3CN$ (4 mg; 0.06 mmol). The reaction solution was kept acidic (pH 4.2~6.2) and was stirred for 90 minutes at room temperature. After evaporation of the solvent, the residue was dissolved in EtOAc, and the organic layer was worked up as usual. TLC separation (benzene-acetone, 5:2) afforded alcohol **22** (3.4 mg): $C_{29}H_{42}O_9$; FAB-MS

535 $(M+H)^+$; UV (EtOH) 236 (22,400); IR (KBr) 3450, 1740; ^1H NMR ($CDCl_3$) 0.75~2.35 (10H, m), 0.98 (3H, d, 16a-H), 1.22 (3H, d, 8a-H), 1.41 (3H, s, 12a-H), 1.76 (3H, s, 18a-H), 2.70~3.30 (6H, m), 3.13 (3H, s, OCH_3), 3.89 (1H, m, 7-H), 4.23 (2H, br s, 22-H), 4.56 (1H, dd, 15-H), 5.35 (1H, dd, 10-H), 5.65 (1H, dd, 9-H), 5.83 (1H, dt, 21-H), 5.97 (1H, d, $J_{19,20}=11.0$, 19-H), 6.53 (1H, dd, $J_{19,20}=11.0$, $J_{20,21}=15.0$, 20-H).

Preparation of 23-*O*-(*p*-Azido)Benzoate **23** from Alcohol **22**

The *p*-azidobenzoate **23** was prepared by treatment of **22** with *p*-azidobenzoic acid in the presence of DMAP and FTNB as described before in 31% yield: $C_{36}H_{45}N_3O_{10}$; HRFAB-MS Calcd for 680.3184 $(M+H)$, Found: 680.3235 $(M+H)^+$; IR (KBr) 3460, 2140, 1740, 1720; ^1H NMR ($CDCl_3$) 0.75~2.40 (10H, m), 1.00 (3H, d, 16a-H), 1.24 (3H, d, 8a-H), 1.42 (3H, s, 12a-H), 1.79 (3H, s, 18a-H), 2.70~3.30 (6H, m), 3.14 (3H, s, OCH_3), 3.89 (1H, m, 7-H), 4.56 (1H, dd, 15-H), 4.88 (2H, m, 22-H), 5.36 (1H, dd, 10-H), 5.62 (1H, dd, 9-H), 5.87 (1H, dt, $J_{20,21}=15.0$, $J_{21,22}=5.0$, 21-H), 5.99 (1H, d, $J_{19,20}=11.0$, 19-H), 6.65 (1H, dd, $J_{19,20}=11.0$, $J_{20,21}=15.0$, 20-H), 7.08 (2H, d, Ph), 8.06 (2H, Ph).

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