Lupane Derivatives from *Lophopetalum wallichii* with Farnesyl Protein Transferase Inhibitory Activity

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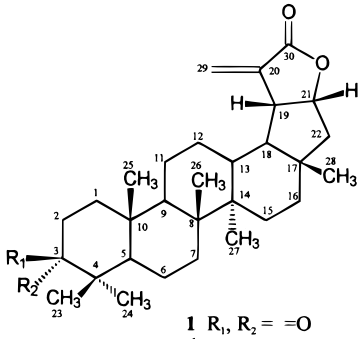
Chloroform-soluble extracts of the stems and of the mixed stems and stem bark of *Lophopetalum wallichii* were found to be inhibitory in a farnesyl protein transferase (FPTase) bioassay system. During the course of activity-guided fractionation, the known lupane-type triterpenes, ochraceolide A (**1**), ochraceolide B (**2**), betulin, and lupeol and the new lupane lactone, dihydro ochraceolide A (**4**), were isolated. The stereochemistry of the epoxide group of ochraceolide B (**2**) was determined by preparation of both epoxide isomers [**2**, and the new semisynthetic derivative, 20-*epi*-ochraceolide B (**3**)] from **1**. The structure of **4** was established by reduction of **1** with sodium borohydride. Compounds **1** and **2** exhibited significant inhibitory activity in the FPTase assay (IC_{50} values of 1.0 and 0.7 μg/mL, respectively). Lupeol was found to be weakly active (IC_{50} 65.0 μg/mL) in this test system, whereas no significant inhibition was detected for betulin or compounds **3** or **4**. When evaluated against a panel of human cancer cells in culture, compounds **1** and **4** were modestly cytotoxic. Compounds **2** and **3** were not active in the panel.

The genus *Lophopetalum* (Celastraceae) is represented by about 18 species of evergreen trees that grow in Cambodia, India, Laos, Malaysia, Myanmar, Thailand, and Vietnam.[1] Only a few species of this genus have been subjected to prior phytochemical investigation, of which the most highly studied is *Lophopetalum toxicum* from the Philippines, whose bark extract is used as an arrow poison.[2] Phytochemical investigation of a *L. toxicum* bark extract with cytotoxic and positive inotropic activity led to the isolation of several cardiac glycosides.[3−5] An examination of the lipids of *Lophopetalum beccarianum* has been carried out,[6] and the triterpenoids friedelin, lupeol, and betulin and the sterol, sitosterol, were reported from a phytochemical study of *Lophopetalum rigidum*.[7] In addition, lupeol and the triterpenoid, pristimerin, were reported from *Lophopetalum wightianum*.[8]

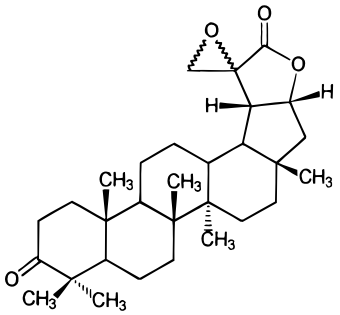
Results and Discussion

In a continuation of our search for novel anticancer agents, $CHCl_3$-soluble extracts of both the mixed stems and stem bark of *L. wallichii* Kurz, and of the stems alone, were found to display significant inhibitory activity in a farnesyl protein transferase (FPTase) assay system.[9] It has been suggested that inhibitors of this enzyme may be considered as potential anticancer agents for tumors in which products of the *ras* oncogene contribute to transformation.[10,11] A number of natural product FPTase inhibitors of fungal or microbial origin have been reported, including actinoplanic acid A,[12] barceloneic acid A,[13] chaetomellic acids A and B,[10,14] cylindrol A,[15] fusidienol,[16] gliotoxin and acetylgliotoxin,[17] manumycin derivatives,[18] pepticinnamin E,[19] several preussomerins and deoxypreussomerins,[20] and zaragozic acids D and D_2,[14,21] although not all are competitive inhibitors with farnesyl pyrophosphate.

In the present investigation, bioassay-directed fractionation of two active extracts led to the isolation of the known lupane derivatives ochraceolide A (**1**),[22] ochraceolide B (**2**),[22] betulin, and lupeol and the new triterpene, dihydroochraceolide A (**4**). Compounds **1**, **2**,



1 R_1, R_2 = =O
4 R_1 = OH, R_2 = H



2 20,29-α-Epoxide
3 20,29-β-Epoxide

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Table 1. ^{1}H-NMR Data of Compounds **2**−**4**[a,b]

proton(s)	compound **2**	**3**	**4**
H-2	2.48 (m)	2.47 (m)	
H-3			3.21 (dd, 10.9, 5.3)
H-18	1.78 (ddd, 11.0, 10.5, 3.5)	1.5 (obsc)	
H-19	2.69 (dd, 10.5, 8.5)	2.65 (t, 9.4)	2.47 (dddd, 10.5, 8.1, 2.0, 1.5)
H-21	5.17 (td, 8.5, 7.7)	5.20 (td, 8.7, 7.3)	4.95 (td, 7.3, 6.6)
H-22β	2.29 (dd, 12.6, 7.7)	2.24 (dd, 12.0, 7.3)	2.17 (dd, 12.8, 7.3)
CH_3-23	1.10 (s)	1.08 (s)	1.09 (s)
CH_3-24	1.05 (s)	1.03 (s)	1.05 (s)
CH_3-25	0.94 (s)	0.95 (s)	0.96 (s)
CH_3-26	1.08 (s)	1.08 (s)	1.10 (s)
CH_3-27	0.99 (s)	0.97 (s)	0.92 (s)
CH_3-28	0.84 (s)	0.84 (s)	0.85 (s)
H-29a	3.24 (d, 6.4)	3.20 (d, 6.0)	6.33 (d, 2.0)
H-29b	3.04 (d, 6.4)	3.17 (d, 6.0)	5.71 (d, 1.5)

[a] In $CDCl_3$ at 300 MHz. [b] Chemical shifts are given in ppm using TMS as an internal reference; signal multiplicity and coupling constants (Hz) are in parentheses; obsc indicates overlapping resonances.

and lupeol were found to significantly inhibit the FPTase test system. Epoxidation of ochraceolide A (**1**) led to the formation of ochraceolide B (**2**) and its 20-epimer, **3**. Characterization of both epoxide isomers permitted the assignment of the stereochemistry to the epoxide group of ochraceolide B (**2**) (see below), which was not determined when this compound was first isolated from *Kokoona ochracea* (Elm.) Merrill (Celastraceae).[22] The structure of dihydroochraceolide A (**4**) was confirmed by reduction of ochraceolide A (**1**) with sodium borohydride. Compounds **1**−**4** have been also evaluated in a panel of human cancer cell lines in the present investigation.

Bioassay-guided fractionation of the $CHCl_3$-soluble extract of the combined stems and stem bark of *L. wallichii*, using a FPTase assay, initially afforded the known lupane-type triterpenoid, ochraceolide A (**1**), exhibiting an IC_{50} value of 1.0 μg/mL. This is the second isolation of this compound, which was initially obtained from *K. ochracea*,[22] a species closely related to *L. wallichii* in the same plant family. Compound **1** was identified by direct comparison of its spectroscopic and physical data with those of an authentic sample. A second active compound obtained in this investigation was identified as ochraceolide B (**2**), with an IC_{50} value of 0.7 μg/mL in the FPTase assay. Compound **2** was also previously isolated from *K. ochracea* and was also identified by comparison of its physical and spectroscopic data with those of an authentic sample from *K. ochracea*.[22]

At the time of its initial isolation, the stereochemistry of the C-20,C-29-epoxide group in ochraceolide B (**2**) was not established. Evaluation of molecular models of the epoxide group of ochraceolide B showed that the distances between both of the H-29 protons and H-19 were quite similar in both isomers. These observations suggested that NOE measurements would not be useful in answering this stereochemical question. However, taking advantage of the availability of sufficient amounts of ochraceolide A (**1**),[22] we have epoxidized this compound following the procedure of Valente and Wolfhagen for the preparation of glycidic esters.[23] Several byproducts of higher R_f by TLC than compound **1** were formed, and only two products were obtained that were more polar than ochraceolide A (**1**) by TLC. The compound with the lowest R_f value (minor product) was identical to the natural epoxide **2**. The other major product **3** showed the same UV, IR, and EIMS spectral data as **2**.

Table 2. ^{13}C-NMR Data of Compounds **2**−**4**[a,b]

carbon	compound **2**	**3**	**4**
1	39.49	39.59	38.64
2	34.05	34.01	27.30
3	218.02	217.77	78.82
4	47.23	47.29	38.82
5	54.59	54.83	55.13
6	19.63	19.55	18.24
7	33.40	33.47	34.14
8	40.83	41.03	40.79
9	49.38	49.92	49.84
10	36.87	36.87	37.09
11	21.39	21.57	20.59
12	26.61	26.72	26.51
13	36.87	37.08	37.59
14	43.01	42.96	42.83
15	26.56	27.35	27.72
16	34.05	34.04	34.59
17	43.48	43.33	43.84
18	48.99	51.38	52.21
19	39.96	41.16	45.04
20	56.97	59.36	139.01
21	82.20	82.14	82.24
22	48.26	47.49	47.75
23	26.77	26.64	27.96
24	21.00	21.02	15.40
25	16.04	16.04	16.06
26	15.74	15.89	15.90
27	14.37	14.60	14.18
28	19.17	18.84	19.35
29	52.13	52.60	124.80
30	174.17	174.90	171.82

[a] $CDCl_3$ at 90.8 MHz. [b] Chemical shifts given in ppm using TMS as internal reference.

The ^{1}H-NMR spectra of **2** and **3** (Table 1) showed very similar profiles. Both epoxide products exhibited similar multiplicities and coupling constants for all proton signals, suggesting no changes in the relative stereochemistry of all the asymmetric carbons bearing hydrogen. However, important differences were observed in the chemical shifts of H-18 and H_2-29. In a NOE-difference NMR experiment with compound **3**, irradiation of the signal corresponding to H-21 at 5.20 ppm afforded enhancements in the signals of H-19 (10%), H-22β (2%), and H_3-28 (4%). These results confirmed the *cis*-fusion of the γ-lactone ring for **3** and also indicated that C-17, C-19, C-20, and C-21 exhibit the same stereochemistry as in compounds **1** and **2**. The ^{13}C-NMR spectral data (Table 2) of **2** and **3** were nearly superimposable, although minor differences were observed in the chemical shifts of C-15, C-18, C-19, C-20,

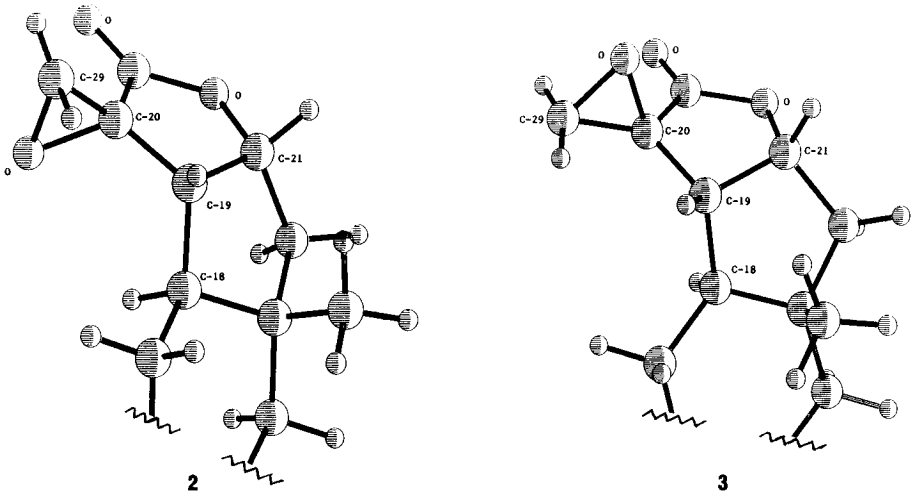

Figure 1. Partial tridimensional views of compounds **2** and **3** generated by PCMODEL.

C-21, and C-30. The assignments of the NMR data of **3** were confirmed using a combination of DEPT, COSY, HETCOR, and selective INEPT experiments[24−26] (Tables 1 and 2). On the basis of all of the above data, compounds **2** and **3** were assigned as a pair of C-20 epimers.

It was still necessary to determine the α- or *β*-stereochemistry of the epoxide group in compounds **2** and **3**. In order to obtain a more accurate three-dimensional representation of both epoxide epimers, the structures of **2** and **3** were generated by the molecular modeling program PCMODEL V 5.0 for Windows, using the MMX force field. The molecular mechanics calculations were performed using a new set of parameters for epoxides described by Podlogar and Raber.[27] Evaluation of the models generated showed that the distance between H-19 and the epoxide proton *trans* to the carbonyl group was 2.65 Å for compound **2** and 2.87 Å for compound **3**, with both interatomic distances falling in the range of an observable NOE effect. In a partial view of the model of the natural epoxide **2** (Figure 1), it can be seen that H-18 and the oxygen of the epoxide group maintain a 1,3-pseudoaxial relationship with an interatomic distance of 2.77 Å. A key observation was the large difference in chemical shift observed for H-18 (1.78 ppm in **2** versus 1.50 ppm in **3**). The deshielding of +0.32 ppm observed for H-18 in the natural epoxide **2** may be rationalized because the oxygen of the epoxide group occurs in an α-orientation. Hence, compound **3** could be assigned as the epimer having the epoxide group in a *β*-orientation. An analogous interaction was observed between H-6 and the oxygen of the epoxide group in a model compound, 3,4-*epi*-11,13-dihydroludartin,[28] where the deshielding observed for H-6 compared with its 3,4-α-isomer was $\Delta\delta$ + 0.24 ppm. This result is similar to that observed for H-18 in the pair of epimers **2** and **3**. In the model of 3,4-*epi*-11,13-dihydroludartin generated with PCMODEL, the interatomic distance between H-6 and the oxygen of the epoxide group was 2.80 Å, very similar to the value observed to H-18 and the corresponding epoxide oxygen in ochraceolide B (**2**). Differences in the ^{1}H-NMR chemical shifts of H-21 and H-19 were not observed between the epimeric pair **2** and **3**. Although H-21 and the oxygen of the epoxide group maintain a 1,3-relationship in **3** (Figure 1), like H-18 and the same oxygen in **2**, the model of **3** shows that the distance between H-21 and this oxygen is 3.16 Å, 0.39 Å more than the distance between the epoxide oxygen and H-18 in compound **2**. This might explain the small difference observed in the proton chemical shift of H-21 in both epoxide isomers. The model of **1**, also generated by PCMODEL, shows that the α-face of the double bond conjugated to the carbonyl group is more hindered to attack by the epoxidizing reagent than the *β*-face. This is in agreement with the higher yield observed for the formation of 20-*epi*-ochraceolide B (**3**) after epoxidation of ochraceolide A (**1**).

The functional group involving the epoxide group and the lactone ring of **2**, which may be termed a glycidic lactone by analogy with glycidic esters, seems to be very rare among natural products. Another example is the sesquiterpene lactone stramonin B, whose absolute configuration was determined by X-ray analysis and by its formal total synthesis.[29]

From a non-FPT-active fraction of the stems of *L. wallichii* we have isolated the novel lupane lactone, dihydroochraceolide A (**4**), as an inactive compound. Compound **4** showed a molecular formula of $C_{30}H_{46}O_3$ by HREIMS, representing two hydrogens more than ochraceolide A (**1**). The determination of the structure of **4** was based mainly on comparison with the spectroscopic data of compound **1**.[22] The IR spectrum of **4** did not show a peak corresponding to the ketone carbonyl group at *ca*. 1700 cm^{-1}, but the signal of the *γ*-lactone carbonyl group was present at 1760 cm^{-1}. In addition, a broad peak characteristic of an alcohol group was observed at 3463 cm^{-1}. The difference of 2 Da in the MS spectra of **4** and **1**, along with evidence from the IR spectra, suggested that compound **1** is the product of the reduction of the carbonyl group at C-3 of ochraceolide A (**1**). This was confirmed from the ^{13}C-NMR spectrum of **4** (Table 2) by the absence of a characteristic signal of the carbonyl group at C-3 at *ca*. 216 ppm and the presence of a new signal of a carbinol carbon at 78.82 ppm, assigned to C-3. Also, the ^{1}H-NMR signals of H-2 (Table 1) were shifted upfield and a new carbinol proton (H-3) appeared at 3.21 ppm (dd, J = 5.3 and 10.9 Hz). The observed vicinal coupling constants involving H-3 and H-2α,*β* are indicative of an axial orientation for H-3 and consequently of the *β*-orientation of the OH group at C-3. The ^{13}C-NMR signals of **4** were assigned by comparison with the chemical shifts reported for lupeol[21,23] and ochraceolide A (**1**). Once the ^{13}C-NMR spectrum (Table 2) was assigned, the proton chemical

Table 3. Potential Inhibition of Farnesyl Protein Transferase (FPTase) Mediated by Compounds **1**−**4**, Lupeol, and Betulin[a]

compound	IC_{50} (μg/mL)
ochraceolide A (**1**)	1.0
ochraceolide B (**2**)	0.7
20-*epi*-ochraceolide B (**3**)	>100
dihydroochraceolide A (**4**)	>100
lupeol	65.0
betulin	>100

[a] Data were determined as described in the Experimental Section.

Table 4. Evalution of the Cytotoxic Potential of Compounds **1** and **4**

human cancer cell line[a]	compound[b] **1**	**4**
BC1	7.9	10.2
HT	8.6	9.6
LU1	16.1	8.9
Mel2	>20	>20
Col2	>20	12.1
KB	>20	>20
KB-V	17.2	>20
A-431	>20	6.8
LNCaP	13.4	14.5
ZR-75-1	4.5	3.0
U373	6.7	9.1

[a] Key to human cell lines: BC1 = breast cancer; HT = fibrosarcoma; LU1 = lung cancer; Mel2 = melanoma; Col2 = colon cancer; KB = oral epidermoid; KB-V = drug-resistant KB; A-431 = epidermoid carcinoma; LNCaP = prostate cancer; ZR-75-1 = hormone-dependent breast cancer; U373 = glioma. [b] EC_{50} values given in μg/mL.

shifts of the methyl groups were determined using the HETCOR experiment. Reduction of ochraceolide A (**1**) with $NaBH_4$ quantitatively produced **4**, showing the stereoselectivity of the reduction of the carbonyl group at C-3 to afford the alcohol group in an equatorial orientation. It is noteworthy that reduction of the ketone carbonyl group at C-3 of ochraceolide A (**1**) led to the complete loss of activity against FPT (Table 3), suggesting that this center is a possible binding site.

Although the $CHCl_3$-soluble extracts of the *L. wallichii* plant parts investigated were not found to be significantly cytotoxic (ED_{50} > 20 μg/mL), the lupane-type triterpenoid lactones **1**−**4** were tested in a panel of human cancer lines. Compounds **1** and **4** were found to be cytotoxic as shown in Table 4, and compounds **2** and **3** were inactive.

On being evaluated in the National Cancer Institute (NCI) 60-cell line human tumor panel,[30] as well as in a newly developed hollow fiber assay,[31] ochraceolide A (**1**) has been selected for *in vivo* evaluation at the NCI in several murine xenograft systems.

Experimental Section

General Experimental Procedures. The ^{1}H-, COSY, and HETCOR NMR spectra were recorded on a Varian XL-300 instrument, using standard Varian programs. The ^{13}C-NMR (BB and DEPT) spectra and selective INEPT NMR experiments were recorded at 90.8 MHz on a Nicolet NMC-360 instrument. For the selective INEPT experiments, data sets of 16 K covering a spectral width of 10 KHz were acquired. Proton pulse widths were calibrated using a sample of acetic acid in 10% C_6D_6 (^{lr}J = 6.7 Hz) in a 5-mm NMR tube.[32] The radiofrequency field strength for the soft pulse was of the order of 25 Hz for these experiments. For the aliphatic protons, 6 Hz was used as $^3J_{CH}$.

Melting points were determined on a Kofler hot-stage apparatus and are uncorrected. Optical rotations were measured with a Perkin-Elmer Model 241 polarimeter. UV spectra were recorded with a Beckman DU-7 spectrophotometer and IR spectra on a Midac Collegian FT-IR interferometer. EI-MS were obtained using a Finnigan MAT 90 instrument.

Plant Material. *L. wallichii* Kurz (Celastraceae) was collected in Thailand in September 1993 at Huey Klongkai, Chiang Mai. A voucher specimen (PA-560) has been deposited in the Royal Forest Herbarium, Bangkok, Thailand.

Extraction and Isolation. The air-dried, powdered mixed stems and stem bark (2 kg) and the stems alone (2 kg) of *L. wallichii* were separately percolated with MeOH (2 × 5 L and 2 × 4 L, respectively). After filtration and evaporation of the solvent, the extracts were suspended in water and successively extracted with *n*-hexane and $CHCl_3$.

The $CHCl_3$-soluble fraction of the mixed stems and stem bark (15.1 g) was subjected to column chromatography over Si gel, eluting with a $CHCl_3$−MeOH gradient of increasing polarity. Fraction 5 (3.45 g), which was eluted with $CHCl_3$−MeOH (7:3), showed significant activity in the FPT assay[9] (IC_{50} 0.7 μg/mL). This fraction was rechromatographed over Si gel using $CHCl_3$ and mixtures of $CHCl_3$−Me_2CO of increasing polarity as eluents. From 24 collected fractions, the best activity was observed in fractions 22 (294 mg) and 23 (300 mg). Fraction 22 was further purified by CC over Si gel, and elution with *n*-hexane−EtOAc (9:1) afforded lupeol (11.2 mg), while elution with *n*-hexane−EtOAc (8:2) afforded a fraction with an IC_{50} of 1.4 μg/mL. Further purification using the same adsorbent and solvent yielded ochraceolide A (**1**) (88.5 mg). From a more polar fraction of this last column, ochraceolide B (**2**) (12 mg) was isolated.

Ochraceolide A (1): white crystals ($CHCl_3$−MeOH) identified by comparison of its spectroscopic data (UV, IR, ^{1}H-NMR, ^{13}C-NMR, EIMS) with an authentic sample of ochraceolide A (**1**).[22]

Ochraceolide B (2): colorless powder with physical and spectroscopic data identical to an authentic sample of ochraceolide B (**2**).[22]

The $CHCl_3$-soluble fraction of the stems (16.9 g) of *L. wallichii* was subjected to column chromatography over Si gel, eluting with *n*-hexane with increasing proportions of EtOAc. The column was monitored by TLC to give 11 fractions. Fraction 7 (1.08 g) showed an IC_{50} of 1.7 μg/mL and was purified twice by column chromatography over Si gel using a *n*-hexane−EtOAc gradient to afford betulin (67.7 mg). Further chromatographic separation of a more polar fraction under the same conditions, followed by column chromatography with Si gel and $CHCl_3$−acetone (98:2) as eluent, resulted in the isolation of compound **4** (11.0 mg).

Dihydroochraceolide A (4): colorless crystals ($CHCl_3$/MeOH); mp 244−246 °C; $[\alpha]_D$ +5.3° (*c* 0.2, $CHCl_3$); UV (MeOH) λ max (log ϵ) 211 (3.65) nm; IR (AgCl) ν max 3465 (OH), 2953, 2863, 1760 (α,β-unsaturated γ-lactone), 1456, 1387, 1256, 1140, 1031, 754 cm^{-1}; ^{1}H-NMR data, see Table 1; ^{13}C-NMR data, see Table 2; EIMS *m/z* $[M]^+$ 454 (49), 436 (96), 421 (40), 292 (24),

314 (24), 300 (17), 233 (20), 219 (18), 207 (41), 189 (100), 187 (33), 175 (42), 147 (45), 135 (81), 123 (48), 121 (74), 107 (64); HR-EIMS *m/z* $[M]^+$ 454.3447 ($C_{30}H_{46}O_3$ requires 454.3446).

Betulin: obtained as a colorless powder and identified by comparison of spectroscopic data (UV, IR, NMR, MS) with those in the literature.[33,34]

Lupeol: white powder, which gave spectroscopic data (UV, IR, NMR, MS) comparable with values published in the literature.[33,34]

Epoxidation of Ochraceolide A (1). *m*-Chloroperbenzoic acid (*m*-CPBA; 50−60%; Aldrich) (150 mg, 0.87 mmol) was added to a solution of ochraceolide A (**1**) (312 mg)[22] in CH_2Cl_2 (100 mL) and the mixture refluxed gently for 75 h, cooled, and shaken with a 10% solution of $NaSO_3$ to destroy excess peroxide. The organic layer was shaken with 5% aqueous $NaHCO_3$ to remove *m*-CPBA and dried over anhydrous Na_2SO_4. The solvent was evaporated under reduced pressure and the residue subjected to column chromatography over Si gel. Fraction 3 (65 mg) was eluted with *n*-hexane−EtOAc (8:2) and was further purified by preparative TLC with the same solvent mixture (developed twice) to give **3** (13.4 mg). Compound **2** (4.8 mg) was isolated in the same way from fraction **4**, which was eluted with *n*-hexane−EtOAc (7:3).

20-*epi*-Ochraceolide B (3): colorless crystals ($CHCl_3$/MeOH); mp dec > 80 °C; $[\alpha]_D$ +9.0° (*c* 0.1, $CHCl_3$); UV (MeOH) λ max (log ϵ) 203 (3.40) nm; IR (AgCl) ν max 2953, 2869, 1782 (γ-lactone), 1703 (ketone), 1458, 1385, 1256, 1119, 932, 752, 663 cm^{-1}; ^{1}H-NMR data, see Table 1; ^{13}C-NMR data, see Table 2; EIMS *m/z* $[M]^+$ 468 (91), 453 (18), 383 (14), 205 (100), 163 (17), 161 (171), 152 (38), 149 (32), 147 (25), 125 (38), 121 (37), 109 (36), 107 (42), 105 (27); HR-EIMS *m/z* $[M]^+$ 468.3234 ($C_{30}H_{44}O_4$ requires 468.3239).

Reduction of Ochraceolide A (1). Ochraceolide A (**1**) (106 mg, 0.23 mmol) was dissolved in 30 mL of $CHCl_3$/MeOH (1:1). The solution was stirred at room temperature on addition of $NaBH_2$ (10 mg, 48 mmol), and the reaction was monitored by TLC. After 1 h, additional $NaBH_4$ (2 mg) was added, and this procedure was repeated three times. After a further 20 min, total conversion of the starting material was observed by TLC. The reaction was stopped by acidification with dilute HCl, washing with H_2O, and extraction with $CHCl_3$. The washed and dried $CHCl_3$ layer was purified by column chromatography over Si gel using *n*-hexane−EtOAc gradient mixtures to give compound **4** (84 mg), which gave comparable spectroscopic data (UV, IR, NMR, MS) to the natural product isolated from *L. wallichii*.

Biological Evaluation Procedures. Farnesyl Protein Transferase (FPTase) Assay. This assay was loosely based on the procedure of Reiss *et al.*[9] [1-^{3}H]-Farensyl pyrophosphate ([^{3}H]-FPP) was purchased from DuPont/NEN (Boston, MA), and streptavidin scintillation proximity assay (SPA) beads were purchased from Amersham (Arlington Heights, IL). Synthetic biotinylated peptide substrate (molecular weight 1006.7; sequence YRASNTSCAIM) and recombinant farnesyl protein transferase (FPTase) were developed in the laboratory of J.M.B. Test samples were initially dissolved in DMSO and then diluted 10-fold with H_2O. Serial dilutions were performed using 10% aqueous DMSO, and 20 μL was added to 96-well plates in duplicate. The highest final concentration tested was 100 μg/mL. Negative control incubations were performed by adding solvent only.

For the assay, reaction mixtures (final volume 100 μL/well) containing the following were prepared in 96-well plates: 20 mM Tris−HCl, pH 7.5, containing 8 mM $MgCl_2$, 12 mM KCl, 0.004% Triton X-100, 0.02 mM biotinylated peptide, 2 mM dithiothreitol, and 0.02 mCi [^{3}H]FPP (0.12 μM). After a preincubation period of 5 min at 37 °C in the presence of test samples (20 μL/well in 10% aqueous DMSO), the reaction was started by the addition of 40 μL of enzyme [final concentration 0.07 μg/mL (1 nM)]. After an additional incubation period of 30 min at 37 °C, the reaction was terminated by the addition of 100 μL of 100 mM sodium citrate buffer, pH 4.0, containing 100 mM $MgCl_2$, 5% bovine serum albumin, 2.2 mg/mL of sodium azide, and 670 mg/mL of SPA beads. The plates were kept at room temperature for 30 min and then directly counted using a MicroBeta 96-well plate scintillation counter.

Data from duplicate determinations were averaged, and negative control values were subtracted from test values. Median inhibitory concentrations (IC_{50} values) were calculated from linearly regressed dose−response plots of percent control activity versus concentration of test substance. In each case, at least five concentrations of test substances were evaluated.

Cytotoxicity Assays. The isolates ochraceolide A (**1**) and dihydroochraceolide A (**4**) were tested against a panel of human cancer cell lines using established protocols (Table 4).[35] Compounds **2** and **3** were inactive (IC_{50} > 20.0 μg/mL) in all panel cell lines.

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