aromatic carbons (δ 120.60; 116.84; 116.15; 113.45), eight quaternary carbons (δ 147.37; 146.63, 44.95; 143.66, 122.77; 122.43; 122.06; 115.38), four methylene carbons (δ 58.43; 57.62; 29.60; 24.48), and a methine carbon (δ 68.21) which arise from the tetrahydroprotoberberine structure, and two protonated olefinic carbons (δ 124.71; 114.42), two quaternary carbons (δ 153.83; 133.45), three methylene carbons (δ 46.96; 41.14; 27.10) and three methyl groups (δ 25.97; 17.89; 16.0) assignable to the geranyl group. The proton signals (H-1') at δ 3.86 and 3.61 display HMBC correlations with the carbons at δ 57.62 (C-8) and δ 58.43 (C-6), respectively, confirming *N*-geranylation, which was also suggested by the NOE correlation between H-8 and H-1'. Assignments of ^{1}H and ^{13}C signals of **16** were made by 1D and 2D (^{1}H-^{1}H COSY, NOESY, HSQC, and HMBC) spectroscopic data. On the basis of these evidences, the structure of **16** was confirmed to be **(±)**-*N*-geranyl-2,3,9,10-tetrademethyltetrahydropalmatininium salt with the B/C-*trans* form as described below (Scheme 3).

Compounds **15** and **16** are stereoisomers of the B/C ring juncture. The chemical shifts of C-6 in **15** and **16** are 51.66 and 58.43 ppm, respectively. The upfield shift of C-6 in **15** compared with **16** is interpreted as being due to steric compression in the B/C-*cis* form because C (6)-H and C (13a)-C (13) bonds are 1, 3-diaxial in the B/C-*cis* form. The chemical shifts at C-13 in **15** and **16** are 34.89 and 29.60 ppm, respectively. The upfield shift of C-13 in **16** compared with **15** arises from steric interaction (1,3-diaxial) between C (13)-H and the *N*-(C-1') bonds in the B/C-*trans* form. Compounds **15** and **16** were established to be **(±)**-*cis*- and *trans*-*N*-geranyl-2,3,9,10-tetrademethyltetrahydropalmatininium salts (Scheme 3). The geometry of the disubstituted olefinic bond (between 2' and 3') was determined to be *E* in both compounds on the basis of the NOE correlation between H-2' and H-4'.

The HPLC of the reaction mixture in geranylation (Scheme 3) of 2,3,10,11-tetrademethylpseudo-tetrahydropalmatine (**17**) is shown in Figure 2 (III). Compound **18**, which corresponds to peak b_6 (Figure 2, III), was purified by prep. HPLC, although a_6 was not purified. The molecular formula of compound **18** was determined to be $C_{27}H_{34}NO_4$ by analysis of its HRSIMS ($[M]^+$, *m/z* 436.2487), which indicates the presence of one geranyl group. The ^{1}H NMR spectrum displays four aromatic proton singlets at δ 6.83, 6.81, 6.70, 6.55, a methine proton at δ 5.07 and four methylene groups [δ 4.39 (1H), 4.34 (1H), 3.85 (1H), 3.78 (1H), 3.63 (1H), 3.25 (1H), 3.15 (1H), and 3.04 (1H)] which arise from the protoberberine skeleton and three methyl proton signals at δ 1.73 (3H, s), 1.67 (3H, s), and 1.32 (3H, s), two olefinic protons at δ 5.44 (1H) and 5.12 (1H), and three methylene groups [δ 3.87 (1H), 3.64 (1H), and δ 2.20 (4H)], assignable to the geranyl group. From a comparison of the ^{1}H NMR spectrum with that of compounds **15** and **16**, compound **18** was postulated to be *N*-geranyl-2,3,10,11-tetrademethyl-pseudotetrahydropalmatinium salt with a B/C-*cis* or B/C-*trans* junction. The ^{13}C NMR spectrum of **18** displays signals corresponding to 27 carbons, including four protonated aromatic carbons

(δ 116.26; 116.15; 114.17; 113.46), eight quaternary carbons (δ 147.62; 146.69; 146.63; 147.39; 122.72; 122.53; 122.05; 118.26), four methylene carbons (δ 61.61; 58.27; 29.83; 24.38), a methine carbon (δ 68.55) arising from the tetrahydroprotoberberine structure, and two protonated olefinic carbons (δ 124.86; 111.41), two quaternary carbons (δ 152.91; 133.27), three methylene carbons (δ 46.69; 41.01; 27.04), and three methyl groups (δ 26.0; 17.91; 16.79) assignable to the geranyl group. Compound **18** was identified as **(±)-*trans*-*N*-**geranyl-2,3,10,11-tetrademethylpseudotetrahydropalmatinium salt (Scheme 3) from comparison of the chemical shifts of C-6 (δ 58.27) and C-13a (δ 68.55) with those (δ 51.66 and 64.34) in **15** (B/C-*cis*) and those (δ 58.43 and 68.21) in **16** (B/C-*trans*). The geometry of the disubstituted olefinic bond (between 2' and 3') was determined to be *E* on the basis of the NOE correlation between H-2' and H-4'. Assignments of ^{1}H and ^{13}C signals of **18** were made by 1D and 2D (^{1}H-^{1}H COSY, NOESY, HSQC, and HMBC) spectroscopic data.

Antimicrobial activity.

The synthetic simple isoquinolines [salsolinol (**1**), *N*-geranylsalsolinol (**2**), *N,N*-digeranylsalsolinol (**3**), *N*-methylsalsolinol (**4**), *N,N*-dimethylsalsolinol (**5**), 7-*O*-geranylsalsolinol (**6**), 6-*O*-geranylsalsolinol (**7**), and 6,7-*O*-digeranylsalsolinol (**8**)], the synthetic 1-benzylisoquinolines [tetrahydropapaveroline (**9**), *N*-geranyl- and *N,N*-digeranyltetrahydropapaveroline (**10** and **11**), *N*-methylpapaveroline (**12**), and *O*, *O*-digeranyl-*N*-methylpapaveroline (**13**)], and the synthetic tetrahydroprotoberberines [2,3,9,10-tetrademethyltetrahydropalmatine (**14**), *cis*- and *trans*-*N*-geranyl-2,3,9,10-tetrademethyltetrahydropalmatinium salts (**15** and **16**), 2,3,10,11-tetrademethylpseudotetrahydropalmatine (**17**), and *trans*-*N*-geranyl-2,3,10,11-tetrademethylpseudotetrahydropalmatininium salt (**18**)] were tested against *Staphylococcus aureus* (Gram-positive) and *Escherichia coli* (Gram-negative) by the liquid dilution method. The minimum inhibitory concentrations (MIC) are presented in Table 1. The results for **1-5** have already been published.[13]

N-Geranylation increases the activity against both bacteria compared with salsolinol (**1**), *N*-methylsalsolinol (**4**), and *N,N*-dimethylsalsolinol (**5**), which are inactive. Notably, *N,N*-digeranylated salsolinol (**3**) displays significant activity (7.8 μg/mL) against *S. aureus*. Thus, according to these data, *N*-quaternization by *N*-geranylation (**3**), not simply *N*-alkylation (**5**), appears to be important for enhanced antimicrobial activity.

Table 1. Antibacterial activity of several 1soquinoline-type alkaloids **1-18**

group	compound	MIC (μg/mL)[a] *Staphylococcus aureus* ATCC25923	MIC (μg/mL)[a] *Escherichia coli* ATCC25922
simple isoquinolines	**1**[b]	> 1000	> 1000
	2[b]	≧ 62.5	≧ 1000
	3[b]	≧ 7.8	≧ 31.3
	4[b]	> 1000	> 1000
	5[b]	> 1000	> 1000
	6	62.5	> 250
	7	62.5	> 250
	8	250	> 250
1-benzylisoquinolines	**9**	> 250	> 250
	10	≧ 250	> 250
	11	≧ 125	> 250
	12	> 250	> 250
	13	≧ 125	> 250
protoberberines	**14**	> 250	> 250
	15	≧ 250	> 250
	16	≧ 250	≧ 250
	17	> 250	> 250
	18	> 250	> 250
	Benzalkonium chlloride	3.9	15.7
	Benzethonium chloride	7.8	7.8

Enhancement of activity against *S. aureus* was observed in *O*-gerany isoquinolines (**6-8**). Enhanced activity was not observed in *N*- or *O*-geranylation of 1-benzylisoquinolines and tetrahydroprotoberberines.

Cytotoxicity evaluation.

The synthetic simple isoquinolines (**1-8**), 1-benzylisoquinolines (**9-13**), and tetrahydroprotoberberines (**14-18**) were assayed for *in vitro* cytotoxicity against five human tumor cell lines, including lung carcinoma (A-549), prostate carcinoma (DU145), epidermoid carcinoma of the nasopharynx (KB), a drug-resistant KB-subline (KBvin), and human promyelocytic leukemia (HL-60). The cytotoxicity data are given as an ED_{50} value for each cell line, the concentration of a compound that caused a 50% reduction in absorbance at 562 nm relative to untreated cells using SRB[10] and MTT[16] and/or WST-8 assays (HL60 is a non-adherent cell line; therefore, the SRB assay could not be used with it), and are shown in Tables 2 and 3. The results for **1-5** have already been published.[13]

The parent salsolinol (**1**), *N*-methylsalsolinol (**4**), and *N,N*-dimethylsalsolinol (**5**) showed no activity in SRB and MTT assays, and *N*-geranylsalsolinol (**2**) showed either weak or no activity against all cell lines in both assays. In comparison, *N,N*-digeranylsalsolinol (**3**) showed especially increased activity against DU-145, KB, and HL-60 cell lines. Compound **3** exhibited the highest potency (1.2 μg/mL) against the HL-60 cell line, and also displayed high activity (0.77 μg/mL) against this cell line in the WST-8 assay (Table 3).[17] Thus, *N*-geranylation, particularly digeranylation, increased cytotoxicity, while *N*-methylation, either mono or di, had no effect. The increase in cytotoxicity was found in *O*- and *O,O*-digeranyl compounds (**6-8**) compared with the parent *N,N*-dimethylsalsolinol (**5**) in the SRB assay, though activity of *O,O*-digeranyl compounds (**8**) was weaker than that of *N,N*-digeranylsalsolinol (**3**). Thus, from these data, *N*-quaternization by *N*-geranylation, not simply *N*-alkylation, appears to be important for enhanced cytotoxicity.

In 1-benzyltetrahydroisoquinoline, *N*- and *N,N*-digeranylations of tetrahydropapaveroline (**9**) increased remarkably the activity against three or four cell lines tested in the SRB assay. Compounds **10** and **11** showed a broad spectrum of activity. It was demonstrated that *N*-geranylation in the 1-benzyltetrahydroisoquinoline-type alkaloids contributes especially to the increase in cytotoxicity. The increase in cytotoxicity by *N,N*-digeranylations (**11**) compared with *N*-geranylation (**10**) was not observed to be distinct from that of the simple isoquinolines.

An increase in cytotoxicity was found in the *O,O*-digeranyl derivative (**13**) of papaveroline (**12**), except for the KBvin cell line in SRB and MTT assays. Compound **13** exhibited high potency (1.49 μg/mL) against the HL-60 cell line, and also displayed high activity (1.24 μg/mL) against this cell line in the WST-8 assay.[17]

Table 2. *In vitro* cytotoxic activity of several isoquinoline-type alkaloids **1-18** against various human tumor cell lines

group[a]	compound	cell line[b] A-549	DU-145	KB	KBvin	HL-60
		ED_{50} (μg/mL)[c]-SRB				
I	**1**[d]	NA[e]	NA	NA	NA	ND[e]
	2[d]	7.75	7.75	10.1	5.64	ND
	3[d]	5.72	3.84	3.28	NA	ND
	4[d]	> 20	> 20	> 20	> 20	ND
	5[d]	> 20	> 20	> 20	> 20	ND
	6	> 20	12.4	12.8	> 20	ND
	7	7.12	13.9	7.70	> 20	ND
	8	8.90	11.5	7.60	> 20	ND
II	**9**	NA	NA	NA	NA	ND
	10	1.31	3.56	3.38	3.60	ND
	11	1.34	2.00	2.35	7.96	ND
	12	11.95	7.46	8.05	5.07	ND
	13	6.25	5.89	5.37	NA	ND
III	**14**	6.05	2.88	5.40	1.45	ND
	15	7.40	3.29	5.87	3.19	ND
	16	3.63	3.26	5.70	1.33	ND
	17	NA	NA	14.6	NA	ND
	18	9.68	2.31	2.90	NA	ND
	Taxol[f]	2.91	1.91	3.10	> 850	ND
		ED_{50} (μg/mL)[c] –MTT[g]				
I	**1**[d]	NA	NA	NA	NA	NA
	2[d]	NA	NA	NA	NA	NA

	3[d]	15.8	3.28	5.70	NA	1.20
II	**12**	NA	NA	NA	NA	12.3
	13	7.50	5.26	6.43	NA	1.49

[a]I: simple isoquinolines, II: 1-benzylisoquinolines, III: protoberberines
[b]A-549: lung carcinoma, DU-145: prostate carcinoma , KB: epidermoid carcinoma of the nasopharynx, KBvin: drug-resistant, HL-60: human promyelocytic leukemia.
[c]Cytotoxicity as ED_{50} for each cell line, the concentration of compound that causes a 50% reduction in adsorbance at 562 nm relative to unreated cells using the SRB or MTT assay. Pure compound is considered to be significantly active when its $ED_{50} < 4.0$ μg/mL.
[d]These data have already been prepared.[13] [e]NA: no activity (if it does not have 50% inhibition at 20 μg/mL, we suggest it has no activity); ND: not determined; [f]ng/mL; [g]Different time of treatment (because of long doubling time in HL-60): 24 hr for A-549, DU-145, KB, and KBvin; 72 hr for HL-60 in MTT assay

In 2,3,9,10-tetrademethyltetrahydropalmatine (**14**), an increase in cytotoxicity by *N*-geranylation (**15**, **16**) of the stereostructure was not observed in almost all cell lines, while in the *N*-geranyl derivative (**18**) with a B/C-*trans* junction of 2,3,10,11-tetrademethylpseudotetrahydropalmatine (**17**), the activity increased, except for the KBvin cell line. Contrary to this, compounds **15** and **16** displayed higher activity than **18** against the HL-60 cell line.

Comparing the activity between the compounds and the cell lines, *N*- and *N,N*-geranyl derivatives (**10** and **11**) of the 1-benzyltetrahydroisoquinolines and *N*-geranyl compound (**16**, tetrahydroprotoberberine) with a B/C-*trans* junction showed high potency (1.31-3.63 μg/mL) in three or four cell lines. The *N,N*- and *N*-geranylated compounds (**3**, simple isoquinoline and **18**, tetrahydroprotoberberine) displayed high activity (2.31-3.84 μg/mL) in DU145 and KB cell lines. 2,3,9,10-Tetrademethyltetrahydroprotoberberine (**14-16**) exhibited high potency (1.33-3.19 μg/mL) in the KBvin cell line, independent of the presence of the geranyl group. In both MTT and WST-8 assays, only two *N,N*- and *O,O*-geranylated compounds (**3**, simple isoquinoline and **13**, 1-benzylisoquinoline) displayed high activity in the HL-60 cell line. It was suggested that the structures of the test samples had a strong effect on the HL-60 cell line.

Table 3. *In vitro* cytotoxic activity of several isoquinolines-type alkaloids**1-4** and **9-18** against HL-60

group	compound	IC_{50} value (μg/mL)-WST-8
simple isoquinolines	**1**	7.17
	2	7.33
	3	0.77
	4	5.09
1-benzylisoquinolines	**9**	6.99
	10	4.78
	11	7.32
	12	3.68
	13	1.24
protoberberines	**14**	>20
	15	8.05
	16	12.38
	17	6.15
	18	16.3

Inhibitory effects on EBV-EA induction.

The Epstein-Barr virus early antigen (EBV-EA) activation assay is considered to be an effective indicator for the evaluation of anti-tumor-promoting activity.[11] The inhibitory effects of simple isoquinolines (**1-8**), 1-benzylisoquinolines (**9-13**), and tetrahydroprotoberberines (**14-18**) on EBV-EA activation induced by 12-*O*-tetradecanoylphorbol-13-acetate (TPA) in Raji cells were examined as a primary screening of anti-tumor-promoting activity.

Table 4. Inhibitory effects of several isoquinoline-type alkaloids **1–18** on TPA Induced EBV-EA activation (100%)[a]

group	compound	concentration (mol ratio/32 pmol TPA) 1000	500	100	10	IC_{50}[b]
		% to control (% viability)				
simple isoquinolines	**1**[c]	12.0 (60)	37.6	79.0	100	384
	2[c]	3.1 (70)	24.3	71.6	97.4	350
	3[c]	0 (70)	20.6	69.3	91.7	296
	4[c]	9.3 (60)	36.8	78.8	100	372
	5[c]	8.7 (60)	35.1	78.0	100	369
	6	8.9 (60)	36.8	69.8	98.7	371
	7	8.0 (60)	36.1	69.0	98.0	369
	8	5.3 (60)	32.6	67.3	96.5	335
1-benzylisoquinolines	**9**	13.9 (60)	37.5	76.9	100	387
	10	11.8 (60)	36.1	75.4	100	380
	11	8.9 (60)	35.0	74.3	100	369
	12	15.0 (60)	39.2	80.6	100	410
	13	9.1 (60)	31.2	72.4	100	323
protoberberines	**14**	0 (60)	25.8	76.8	96.9	321
	15	0 (60)	23.2	74.2	94.3	307
	16	0 (60)	23.7	74.8	94.7	308
	17	0 (60)	23.7	71.5	94.0	305
	18	0 (60)	23.2	71.1	93.8	303
	Ginsenoside-Rg1	0 (80)	32.5	72.6	91.0	310
	β-Carotene	9.1 (60)	34.3	82.7	100	400

[a]Values represent percentages relative to the positive control value. TPA (32 pmol, 20 ng) = 100%. Values in parentheses are viability percentages of Raji cell. [b]IC_{50} represents the mol ratio to TPA that inhibits 50% of positive control (100%) activated with 32 pmol of TPA.

[c]These data have been published.[13]

The inhibitory effects of the test compounds on TPA-induced EBV-EA activation, their effects on the viability of Raji cells, and the 50% inhibitory concentration (IC_{50}) values are shown in Table 4. The results for **1-5** have already been published.[13]

All simple isoquinolines (**1-8**) displayed stronger inhibition (IC_{50} 296-384) than that of the reference β-carotene (IC_{50} 400), which has been studied extensively in cancer chemoprevention using animal models.[18] The inhibitory activity was more increased by geranylation than by methylation on nitrogen (compared with **3** and **5**). Therefore, *N*-geranylation but not *N*-dimethylation appears to be important for enhanced activity. *N*,*N*-Digeranylsalsolinol (**3**) displayed the strongest inhibition (IC_{50} 296), and its activity was higher than that of ginsenoside-Rg1, which is known as a strong anti-tumor-promoter.[19]

The 6,7-*O*-digeranylated derivative (**8**) had increased activity (IC_{50} 335) compared with the parent compound (**5**), though 6- and 7-*O*-geranylation (**6** and **7**) scarcely affected the activity. Thus, these compounds, especially *N*,*N*- and *O*,*O*-digeranylated derivatives, appear to be useful leads for further development of potential cancer chemopreventive agents.

There was little increase in inhibition by *N*-, and *N*,*N*-geranylation (**10** and **11**) of tetrahydropapaveroline (**9**). Increased inhibition (IC_{50} 323) was observed in *O*, *O*-geranylation (**13**) of papaveroline (**12**). All tetrahydroprotoberberines (**14-18**) displayed strong inhibition (IC_{50} 303-321) comparable to ginsenoside-Rg1 (IC_{50} 310), independent of whether the compound has the geranyl group or not. They might be valuable antitumor promoters. In 2, 3, 9, 10-tetrademethyltetrahydropalmatine (**14**), there was little increase in inhibition by *N*-geranylation (**15**, **16**) independent of the stereostructure.

In simple isoquinolines and 1-benzylisoquinolines, digeranylated derivatives (**3**, **8**, and **13**) displayed potent activity, suggesting that relative lipophilicity of the geranyl groups may contribute to the inhibitory effect.

Free radical scavenging activity.

Several human illnesses, such as cancer, diabetes, atherosclerosis, etc., can be linked to the damaging action of reactive free radicals.[20] The ability of three types of compounds (**1–18**) to scavenge 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radicals was examined; the results are presented in Table 5. The results for **1-3** have already been published[13]. To evaluate the free radical scavenging activity of these compounds, the concentration required to scavenge DPPH free radicals by 50% (SC_{50}) was determined. The antioxidant α-tocopherol was used as a reference compound. Compounds **1-3** displayed similar potency and were more active than α-tocopherol. 6-*O*- or 7-*O*-Geranylation (**6** or **7**) of **5** reduced the activity and 6,7-*O*-digeranylation (**8**) further reduced the activity.

Table 5. Radical scavenging activity of several isoquinoline-type alkaloids **1-18**

group	compound	SC_{50} (μM)[a]
simple isoquinolines	**1**[b]	12.0
	2[b]	17.0
	3[b]	11.5
	4	32.1
	5	31.3
	6	40.7
	7	41.1
	8	57.7
1-benzylisoquinolines	**9**	2.64
	10	7.81
	11	6.47
	12	2.95
	13	9.94
protoberberines	**14**	5.53
	15	8.39
	16	3.81
	17	6.04
	18	5.36
	α-tocopherol	24.3

[a]The compound concentration showing radical scavenging efficacy of 50% was defined as SC_{50}.
[b]These data have already been prepared.[13]

All 1-benzyltetrahydroisoquinolines (**9-13**) are more active than α-tocopherol. *N*-Geranylation (**10**, **11**) of 1-benzyltetrahydroisoquinoline (**9**) reduced the activity. The activity also decreased by *O*,*O*-digeranylation (**13**) of papaveroline (**12**). All tetrahydroprotoberberines (**14-18**) proved to be more active than α-tocopherol; increased activity by *N*-geranylation was small.

In all three types of isoquinoline alkaloids, the activity was reduced by *N*- or *O*-geranylation. Obviously the phenolic hydroxyl groups on the aromatic rings strongly influenced the activity, without an affect of the geranyl group.

Antimalarial activity.

The simple isoquinolines (**1-3**), 1-benzylisoquinolines (**12**, **13**), and tetrahydroprotoberberines (**14**, **16-18**) were tested *in vitro* against human malaria parasite, *Plasmodium falciparum* FCR-3. The antimalarial activity of each compound was determined as a percentage of reduction compare of control. The compound concentration required to inhibit cell growth by 50% was expressed as EC_{50}. To evaluate the toxicity of the compounds for mammalian cells, the concentration causing a 50% growth reduction (IC_{50}) of mouse mammary FM3A cells, a model of the host, was determined. The IC_{50}/EC_{50} ratios for the compounds were calculated as an evaluation of antimalarial activity. The results are presented in Table 6. In three types of isoquinoline derivatives, *N*-geranylation increased the inhibitory activity; however, the geranylated derivatives of the simple isoquinolines and 1-benzylisoquinolines showed only slight inhibitory activity and no selectivity. Only *N*-geranyl derivatives (**16** and **18**) of the tetrahydroprotoberberines inhibited *P. falciparum* with EC_{50} values in the order of 10^{-7} M. This is an increase antimalarial activity compared with the parentcompounds (**14** and **17**), and their selectivity indexes were > 15. Compounds **16** and **18** were potent antimalarial agents with higher selectivity indexes compared with the other test compounds.

In addition, *N*-geranyl derivatives, **16** and **18**, it is noted that the selectivity of the compounds (selectivity; >15) was comparable to that of mefloquine (selectivity; 90). It means that these compounds have safety for human clinical treatment of malaria if these compounds developed new antiamalrial drug.

Table 6. *In vitro* antimararial activity of several isoquinoline-type alkaloids **1-3**, **12-14**, and **16-18**

		50 % inhibitory concentration (μM)[a]		selectivity index[b]
group	compound	*Plasmodium falciparum* FCR-3 EC_{50} (growth %)	Mouse mammary cells FM3A IC_{50} (growth %)	IC_{50} / EC_{50}
simple isoquinolines	**1**	>27 (100 %)	9.0	-
	2	5.3	0.08	-
	3	3.4	0.45	-
1-benzylisoquinolines	**12**	5.1	2.9	-
	13	3.6	1.5	-
protoberberines	**14**	>17 (100 %)	>8.3 (73 %)	-
	16	0.33	>4.9 (69 %)	>15
	17	9.5	8.1	-
	18	0.53	>7.7 (69 %)	>15
	Mefloquine	0.032	2.9	91

[a]The 50 % inhibitory concentration was defined by comparison with drug-free controls incubated under same condition.

[b]In vitro selectivity index was estimated from the ratio (IC_{50} / EC_{50}) of the drug concentrations necessary to inhibit the growth rate of cells to 50 % of the growth value between the malaria parasites
and mouse mammary FM3A cells which served as a model host.

Table 7. Anti-HIV activity of several isoquinoline-type alkaloids **1-18**

group	compound	IC_{50}(μg/mL)[a]	EC_{50} (μg/mL)[b]
simple isoquinoline type	**1**[c]	>25	>2.5
	2[c]	20.23	>2.5
	3[c]	10.68	>2.5
	4	>25	>2.5
	5	>25	>25
1-benzylisoquinoline type	**9**	19.69	>2.5
	10	24.59	>2.5
	11	18.02	>2.5
	12	>25	>2.5
	13	17.95	>2.5
protoberberine type	**14**	>25	>2.5
	15	19.84	>2.5
	16	>25	>25
	17	18.13	22.54
	18	>25	>25
	AZT[d]	500	0.014

[a]The agent concentration that inhibited H9 cell growth by 50%. [b]The agent concentration that inhibited viral replication in H9 cell by 50%. [c]These data have already been prepared.[13]

[d]Azidothymidine

Anti-HIV activity.

The isoquinoline alkaloids (**1-4** and **9-18**) were tested against HIV-1 replication in H9 lymphocytes in order to evaluate their anti-HIV activity. However, none of them displayed anti-HIV activity (Table 7). Interestingly, results in the anti-HIV assay did not parallel those in the antimicrobial and cytotoxicity assays.

Conclusions.

In summary, three types of isoquinoline alkaloids (**1-18**) were tested for antimicrobial, cytotoxic, anti-malarial, anti-oxidant, and anti-HIV activities, as well as inhibitory activity against Epstein-Barr virus early antigen (EBV-EA) activation induced by 12-*O*-tetradecanoylphorbol-13-acetate (TPA) in Raji cells. *N*- or *O*-Geranylation contributed to increased potency in four types of activities except anti-HIV and anti-oxidant. *N,N*-Geranylation of salsolinol (simple isoquinoline, **1**) strongly increased the potency in antimicrobial activity. *N,N*- and *N*-Geranylations of tetrahydropapaveroline (1-benzylisoquinoline, **9**) strongly enhanced cytotoxic activity. *N,N*-Geranylation of **1** and *N*-geranylation of **17** (2,3,10,11-oxygenated protoberberine) increased the same activity to a lesser extent. *N,N*-Geranylation of **1** and *O,O*-geranylation of papaveroline (1-benzylisoquinoline, **11**) strongly increased cytotoxic activity against the HL-60 cell line. *N,N*-Geranylsalsolinol (**3**) showed potent inhibitory effects on EBV-EA induction compared with those of the parent compound (**1**). *O,O*-Geranylation of **5** (simple isoquinoline) and *N,N*-geranylation of **11** (1-benzylisoquinoline) enhanced the inhibitory activity. The protoberberines (**14-18**) tested also displayed strong inhibitory activity. *N*-Geranylation of 2,3,9,10- and 2,3,10,11-oxygenated protoberberines increased the antimalarial activity. Among the tested biological activities of the isoquinolines **1-18**, *N,N*-geranylation of salsolinol (**1**) strongly increased potency in three assays, antimicrobial and cytotoxic activities and inhibitory effects on EBV-EA induction, while *N*-geranylation increased the same activities to a lesser extent. However, *N,N*- and *N*-methylation did not increase the activities in these assays. These simple *N*-geranylated isoquinolines also have free radical scavenging activity. These findings indicate that the *N*-geranyl group plays an important role in mediating these biological activities.

Compound **3** shows antimicrobial, cytotoxic, and inhibitory effects on EBV-EA induction, compounds **10**, **11**, **13**, and **18** are cytotoxic, **8**, **13**, and **14-18** inhibit EBV-EA induction, and **16** and **18** display antimalarial activity. In the present studies, we have identified new biologically active *N*- or *O*-geranylated isoquinolines, which may be considered as lead structures for developing potential chemotherapeutic agents. It was first suggested that the addition of a geranyl residue to isoquinoline skeletons may contribute to the enhancement of the biological activities of isoquinoline alkaloids.

EXPERIMENTAL

General procedures. Conventional ^{1}H NMR, NOESY, COSY, HMBC, and HMQC spectra were obtained on a Varian VXR-500 spectrometer (^{1}H: 500 MHz) using CD_3OD solvent, except where noted, with TMS as int. standard. ^{13}C NMR and DEPT spectra were measured on a Varian VXR-500 spectrometer (125 MHz). Mass spectra were determined on a Hitachi M-4100 instrument at 75 eV. The secondary ion mass spectra (SIMS) were measured using glycerol as matrix. HPLC and prep. HPLC analyses were performed using a Hitachi M-6200 intelligent pump (1 mL/min) and Hitachi M-6250 or Jasco PU-2089 intelligent pump (6 mL/min), respectively, and a Hitachi L-4000 UV detector (280 nm). Cosmosil 5C18-AR reversed-phase column of small (4.6 i.d. X 150 mm) and large (20 i.d. X 250 mm) sizes were used for HPLC and prep. HPLC, respectively. Analyses with a Hitachi HPLC system were made using a solvent system, (A) 0.1M NH4OAc (0.05% TFA) / (B) MeOH (0.05% TFA) under the following gradient conditions: A/B, initial (75/25), 10 min (50/50), 30 min (20/80) or initial (80/20), 30 min (0/100) or initial (80/20), 20 min (0/100) (flow rate 1 mL/min). Prep. HPLC analyses for purification were performed using a solvent system, (A) H_2O (0.05% TFA) / (B) MeOH (0.05% TFA) under the following gradient conditions: A/B 50/50 to 0/100, 30 to 60 min (flow rate 6 mL/min). (±)-Tetrahydropapaverine hydrochloride and azidothymidine (AZT) were purchased (Sigma). (±)-Salsolinol (**1**),[8] (±)-carnegine,[9] (±)-*N*-methylpapaveroline (**11**),[9] (±)-2,3,9,10-tetrademethyl-tetrahydropalmatine (**14**),[9] and (±)-2, 3, 10, 11-tetrademethylpseudotetrahydropalmatine (**17**)[9] have previously been prepared.

Optimized geometry and molecular orbital were calculated by the DFT Method (Density Function Theory) using the Materials Studio DMol3 package of Accelrys Inc.[14,15] First, optimized geometry was obtained using the Perdew-Wong LDA functional (PWC)[21] and double numerical plus d-functional (DND) basis set. Second, the optimized geometry obtained was further calculated for molecular orbitals using the Becke exchange plus Lee-Young-Parr correlation (BLYP)[22, 23] and the double numerical plus polarization (DNP) basis set.

Preparations of (±)-*N*- and (±)-*N*,*N*-geranylsalsolinol (2 and 3). To a stirred suspension of sodium hydride (245 mg, 10.2 mmol) in DMF (15 mL) at room temperature under N_2 (±)-salsolinol hydrochloride[8] (**1**, 1 g, 4.65 mmol) was added by portions followed by a catalytic amount of hydroquinone. The mixture was stirred for 30 min. Geranyl bromide (953 mg, 4.39 mmol) was added dropwise and the mixture was stirred for 2 h. NaH (245 mg, 10.2 mmol) and geranyl bromide (953 mg, 4.39 mmol) were added again, and the mixture was stirred for 2 h. After decomposition of excess NaH with MeOH (1 mL), the mixture was poured onto ice-water and extracted with Et_2O followed by $CHCl_3$. Et_2O and $CHCl_3$ were separately dried and evaporated. The $CHCl_3$ extract was subjected to prep.

HPLC [0.1M NH_4OAc (0.05% TFA) / MeOH (0.05% TFA) (A/B) initial 60/40, 0/100 (30 min)] to give (±)-*N*-geranylsalsolinol (**2**, 205.7 mg, yield 10.3%) and (±)-*N*,*N*-geranylsalsolinol (**3**, 329.9 mg; yield 12.6%) as trifluoroacetate. **2**: SIMS *m/z* $[M + H]^+$ 316; HRMS *m/z* $[M + H]^+$ 316.2257 ($C_{20}H_{30}NO_2$ requires 316.2271). **3**: SIMS *m/z* $[M]^+$ 452; HRMS *m/z* $[M]^+$ 452.3527 ($C_{30}H_{46}NO_2$ requires 452.3523). The ^1H and ^{13}C NMR spectral data of these compounds have been previously presented.[13]

Preparation of (±)-*N*-methylsalsolinol (4). Carnegine-HCl (500 mg, 1.94 mmol) in 47% HBr (2 ml) was refluxed for 2 h at 130° in an oil bath. The solvent was evaporated *in vacuo*, the residue in MeOH was crystallized to give (±)-*N*-methylsalsolinol-HBr (**4**, 398.5 mg, yield 64.8%). SIMS *m/z* $[M + H]^+$ 194; HRMS *m/z* $[M + H]^+$ 194.1201 ($C_{11}H_{16}NO_2$ requires 194.1176). The ^1H and ^{13}C NMR spectral data of **4** have been previously presented.[13]

Preparation of (±)-*N*,*N*-dimethylsalsolinol (5). A solution of carnegine-HCl (3.1 g, 12.06 mmol) and CH_3I (1 ml) in MeOH (25 ml) and $(Me)_2CO$ (25 ml) in a glass-stoppered bottle was heated for 75 min at 110 °C in a oil bath. The solvent was evaporated *in vacuo*, the residue was crystallized in $(Me)_2CO$-Et_2O to give *N*-methylcarnegine iodide (4.75 g, yield 86.5%). The iodide (3.8 g) in 47% HBr (10 ml) was refluxed for 1 h at 140 °C in an oil bath. The solvent was evaporated *in vacuo*, the residue was crystallized in $(Me)_2CO$-Et_2O to give (±)-*N*,*N*-dimethylsalsolinol bromide (**5**, 2.97 g, yield 98.5%). SIMS *m/z* $[M]^+$ 208; HRMS *m/z* $[M]^+$ 208.1350 ($C_{12}H_{18}NO_2$ requires 208.1336). The ^1H and ^{13}C NMR spectral data of **5** have been previously presented.[13]

Preparations of (±)-7-*O*-geranyl-, (±)-6-*O*-geranyl-, and (±)-6,7-*O*-digeranyl-*N*,*N*-dimethylsalsolinol (6-8). To a stirred suspension of NaH (260 mg, 10.8 mmol) in DMF (15 mL) at rt under N_2 (±)-*N*,*N*-dimethylsalsolinol hydrobromide (**5**, 1 g, 3.47 mmol) was added portionwise followed by a catalytic amount of hydroquinone. The mixture was stirred for 30 min. Geranyl bromide (1 g, 4.61 mmol) was added dropwise and the whole mixture was stirred for 3 h. After decomposition of excess NaH with MeOH (1 mL), the mixture was poured onto ice-water and extracted with $CHCl_3$. The combined organic layer was dried and evaporated. The $CHCl_3$ extract was subjected to prep. HPLC [0.1M NH_4OAc (0.05% TFA)/MeOH (0.05% TFA) (A/B) initial 100/0, 0/100 (540 min)] to give (±)-7-*O*-geranyl-*N*,*N*-dimethylsalsolinol (**6**, 102.2 mg, yield 7.7%), (±)-6-*O*-geranyl-*N*,*N*-dimethylsalsolinol (**7**, 98.8 mg, yield 7.4%) and (±)-6,7-*O*-digeranyl-*N*,*N*-dimethylsalsolinol (**8**, 154.2 mg, yield 8.9%) as trifluoroacetates. **6**: ^1H NMR (500 MHz, CD_3OD) δ 6.68 (1H, s, H-5), 6.74 (1H, s, H-8), 5.48 (1H, t, J = 6.5 Hz, H-2′), 5.09 (1H, t, J = 6.5 Hz, H-6′), 4.62 (2H, d, J = 6.5 Hz, H-1′), 4.57 (1H, q, J = 6.5 Hz, H-1), 3.77 (1H, dt, J = 12.5, 8.0 Hz, Hax′-3), 3.55 (1H, dt, J = 12.5, 5.0 Hz, Heq′-3), 3.17 (3H, s, ax′NMe), 3.14 (3H, s, eq′NMe), 3.08 (2H, m, H-4), 2.12 (2H, m, H-5′), 2.09 (2H, m, H-4′), 1.75 (3H, s, 3′-Me), 1.69 (3H, d, J = 6.5 Hz, Me-1), 1.65 (3H, s, 8′), 1.59 (3H, s, 7′-Me); ^{13}C NMR (125 MHz,

CD_3OD) δ 148.79 (C-6), 147.83 (C-7), 142.30 (C-3′), 132.63 (C-7′), 124.90 (C-6′), 124.39 (C-1a), 122.17 (C-4a), 120.94 (C-2′), 116.01 (C-5), 113.25 (C-8), 69.51 (C-1), 67.06 (C-1′), 56.86 (C-3), 51.50 (eq′NMe), 50.11 (ax′NMe), 40.61 (C-4′), 27.41 (C-5′), 25.84 (C-8′), 24.30 (C-4), 18.63 (C-1-Me), 17.72 (7′-Me), 16.69 (3′-Me); SIMS *m/z* $[M]^+$ 344; HRMS *m/z* $[M + H]^+$ 344.2606 ($C_{22}H_{34}NO_2$ requires 344.2588), *m/z* $[M\text{-}C_{10}H_{16}]^+$ 208.1355 ($C_{12}H_{18}NO_2$ requires 208.1338) ; **7**: ^1H NMR (500 MHz, CD_3OD) δ 6.79 (1H, s, H-5), 6.66 (1H, s, H-8), 5.50 (1H, m, H-2′), 5.10 (1H, m, H-6′), 4.63 (2H, d, *J* = 6.5 Hz, H-1′), 4.55 (1H, q, *J* = 6.5 Hz, H-1), 3.78 (1H, dt, *J* = 12.5, 7.5 Hz, Hax′-3), 3.56 (1H, dt, *J* = 12.5, 6.0 Hz, Heq′-3), 3.17 (3H, s, ax′NMe), 3.13 (3H, s, eq′NMe), 3.12 (2H, m, H-4), 2.12 (2H, m, H-5′), 2.07 (2H, m, H-4′), 1.75 (3H, s, 3′-Me), 1.67 (3H, d, *J* = 6.5 Hz, Me-1), 1.65 (3H, s, Me-8′), 1.60 (3H, s, 7′-Me); ^{13}C NMR (125 MHz, CD_3OD) δ 148.62 (C-6), 147.90 (C-7), 142.26 (C-3′), 132.63 (C-7′), 124.90 (C-6′), 124.39 (C-1a), 122.17 (C-4a), 120.94 (C-2′), 116.01 (C-5), 113.25 (C-8), 69.36 (C-1), 66.86 (C-1′), 57.19 (C-3), 51.58 (eq′NMe), 49.80 (ax′NMe), 40.62 (C-4′), 27.39 (C-5′), 25.86 (C-8′), 24.51 (C-4), 18.36 (C-1-Me), 17.74 (7′-Me), 16.67 (3′-Me); SIMS *m/z* $[M]^+$ 344; HRMS *m/z* $[M + H]^+$ 344.2603 ($C_{22}H_{34}NO_2$ requires 344.2588), *m/z* $[M\text{-}C_{10}H_{16}]^+$ 208.1317 ($C_{12}H_{18}NO_2$ requires 208.1338); **8**: ^1H NMR (500 MHz, CD_3OD) δ 6.83 (1H, s, H-5), 6.79 (1H, s, H-8), 5.48 (2H, m, H-2′, H-2′′), 5.10 (2H, m, H-6′, H-6′′), 4.59 (2H, m, H-1′′), 4.58 (2H, m, H-1′), 4.54 (1H, q, *J* = 6.5 Hz, H-1), 3.79 (1H, dt, *J* = 12.5, 8.0 Hz, Hax′-3), 3.57 (1H, dt, *J* = 12.5, 5.0 Hz, Heq′-3), 3.17 (3H, s, ax′NMe), 3.15 (3H, s, eq′NMe), 3.14 (2H, m, H-4), 2.12 (4H, m, H-5′, H-5′′), 2.07 (4H, m, H-4′, H-4′′), 1.742 (3H, s, 3′′-Me), 1.736 (3H, s, 3′-Me), 1.69 (3H, d, *J* = 6.5 Hz, Me-1), 1.66 (6H, s, 8′, 8′′), 1.60 (6H, s, 7′-Me, 7′′-Me); ^{13}C NMR (125 MHz, CD_3OD) δ 150.79 (C-6), 149.78 (C-7), 142.58(C-3′′), 142.56 (C-3′), 132.68 (C-7′), 125.09 (C-1a), 124.96 (C-6′), 122.33 (C-4a), 120.94 (C-2′′), 120.85 (C-2′), 115.05 (C-5), 114.41 (C-8), 69.60 (C-1), 67.38 (C-1′), 67.04 (C-1′′), 56.83 (C-3), 51.55 (eq′NMe), 50.25 (ax′NMe), 40.66 (C-4′, C-4′′), 27.48 (C-5′), 27.44 (C-5′′), 25.93 (C-8′), 24.56 (C-4), 18.63 (C-1-Me), 17.81 (7′-Me), 16.77 (3′-Me), 16.74 (3′′-Me); SIMS *m/z* $[M]^+$ 480; HRMS *m/z* $[M]^+$ 480.3858 ($C_{32}H_{50}NO_2$ requires 480.3837), *m/z* $[M\text{-}C_{10}H_{16}]^+$ 344.2586 ($C_{22}H_{34}NO_2$ requires 344.2588).

Preparation of (±)-tetrahydropapaveroline (9). A solution of (±)-tetrahydropapaverine hydrochloride (Sigma, 2 g, 5.26 mmol) in 47% HBr (10 ml) was refluxed for 2.5 h. The solvent was evaporated *in vacuo*. The crystalline product was recrystallized from MeOH to give (±)-tetrahydropapaveroline hydrobromide **9** (1.84 g, yield 95.1%) which was identified by comparing its ^1H NMR and HPLC with data of an authentic sample.

Preparations of (±)-*N*- and (±)-*N*,*N*-digeranyltetrahydropapaveroline (10 and 11). To a stirred suspension of NaH (65 mg, 2.71 mmol) in DMF (15 mL) at rt under N_2 (±)-tetrahydropapaveroline hydrobromide (**9**, 1 g, 2.72 mmol) was added portionwise followed by a catalytic amount of

hydroquinone. The mixture was stirred for 30 min. Geranyl bromide (590 mg, 2.72 mmol) was added dropwise, and the mixture was stirred for 10 h. Geranyl bromide (295 mg, 1.36 mmol) was added dropwise and the mixture was further stirred for 12 h. After decomposition of excess NaH with MeOH (1 mL), the mixture was poured onto ice-water and extracted with Et_2O followed by $CHCl_3$. The Et_2O and $CHCl_3$ phases were separately dried and evaporated. The $CHCl_3$ extract was subjected to prep. HPLC [0.1M NH_4OAc (0.05% TFA)/MeOH (0.05% TFA) (A/B) initial 80/20, 0/100 (60 min)] to give (±)-*N*-geranyltetrahydropapaveroline (**10**, 274 mg, yield 18.7%) and (±)-*N*,*N*-digeranyltetrahydropapaveroline (**11**, 175 mg, yield 9.5%) as trifluoroacetate. **10**: ^{1}H NMR (500 MHz, CD_3OD) δ 6.75 (1H, d, *J* = 8.5 Hz H-5′), 6.65 (1H, s, H-5), 6.58 (1H, brs, H-2′), 6.50 (1H, brd, *J* = 8.5 Hz, H-6′), 6.18 (1H, s, H-8), 5.26 (1H, m, H-2′′), 5.12 (1H, m, H-6′′), 4.42 (1H, t, *J* = 6.5 Hz, H-1), 3.83 (1H, dd, *J* = 13.0, 8.5 Hz, H-1′′), 3.71 (1H, m, H-1′′), 3.68 (1H, m, H-3), 3.38 (1H, m, H-3), 3.14 (1H, m, H-9), 3.04 (1H, m, H-9), 2.97 (2H, m, H-4), 2.18 (4H, s, H-4′′, H-5′′), 2.12 (2H, m, H-5′), 2.07 (2H, m, H-4′), 1.70 (3H, s, Me-8′′), 1.64 (3H, s, 7′′-Me), 1.57 (3H, s, 3′′-Me); ^{13}C NMR (125 MHz, CD_3OD) δ 149.65 (C-3′′), 147.30 (C-6), 146.92 (C-3′), 146.04 (C-4′), 145.65 (C-7), 133.29 (C-7′′), 127.63 (C-1′), 124.56 (C-6′′), 121.93 (C-4a), 121.87 (C-1[a], C-6′), 117.67 (C-2′), 116.76 (C-5′), 116.12 (C-5), 115.75 (C-8), 114.15 (C-2′′), 63.01 (C-1), 51.86 (C-1′′), 44.80 (C-3), 40.74 (C-4′′, C-9), 27.14 (C-5′′), 25.94 (C-8′′), 22.90 (C-4), 17.84 (7′′-Me), 16.80 (3′′-Me); SIMS *m/z* $[M + H]^+$ 424; HRMS *m/z* $[M + H]^+$ 424.2494 ($C_{26}H_{34}NO_4$ requires 424.2482); **11**: ^{1}H NMR (500 MHz, CD_3OD) δ 6.67 (1H, d, *J* = 8.0 Hz H-5′), 6.64 (1H, s, H-5), 6.38 (1H, brs, H-2′), 6.30 (1H, dd, *J* = 8.0, 2.0 Hz, H-6′), 5.89 (1H, s, H-8), 5.61 (1H, d, *J* = 7.0 Hz H-2′′′), 5.42 (1H, t, *J* = 7.0 Hz, H-2′′), 5.15 (1H, m, H-6′′′) 4.99 (1H, m, H-6′′), 4.42 (1H, dd, *J* = 9.5, 3.0 Hz, H-1), 4.20 (1H, dd, *J* = 14.5, 7.0 Hz, H-1′′′) 4.13 (1H, dd, *J* = 14.5, 7.0 Hz, H-1′′′), 3.96 (1H, dd, *J* = 14.5, 9.0 Hz, H-1′′), 3.78 (1H, dd, *J* = 14.5, 6.0 Hz, H-1′′), 3.68 (1H, m, Hax′-3), 3.53 (1H, m, Heq′-3), 3.45 (1H, dd, *J* = 13.5, 3.5 Hz, H-9), 2.88 (1H, dd, *J* = 13.5, 9.5 Hz, H-9), 2.97 (2H, m, H-4), 2.28 (4H, s, H-4′′′, H-5′′′), 2.18 (4H, s, H-4′′, H-5′′), 2.07 (2H, m, H-4′), 1.85 (3H, s, 3′′′-Me), 1.69 (3H, s, Me-8′′), 1.68 (3H, s, Me-8′′′), 1.65 (3H, s, 7′′′-Me), 1.62 (3H, s, 7′′-Me) , 1.46 (3H, s, 3′′-Me); ^{13}C NMR (125 MHz, CD_3OD) δ 151.18 (C-3′′), 150.09 (C-3′′′), 147.52 (C-6), 146.65 (C-3′), 145.82 (C-4′), 145.24 (C-7), 133.37 (C-7′′), 133.32 (C-7′′′), 127.88 (C-1′), 124.70 (C-6′′), 124.65 (C-6′′′), 121.42 (C-4a), 122.52 (C-1a), 122.25 (C-6′), 118.04 (C-2′), 116.51 (C-5′), 115.85 (C-5), 116.81 (C-8), 112.11 (C-2′′, C-2′′′), 70.72 (C-1), 58.30 (C-1′′′), 55.79 (C-1′′), 52.01 (C-3), 41.06 (C-4′′, C-4′′′), 38.32 (C-9), 27.14 (C-5′′′), 27.05 (C-5′′), 25.98 or 25.99 (C-8′′or C-8′′′), 24.0 (C-4), 17.87 or 17.89 (7′′-Me or 7′′′-Me), 17.28 (3′′′-Me), 16.90 (3′′-Me);SIMS *m/z* $[M]^+$ 560; HRMS *m/z* $[M]^+$ 560.3746 ($C_{36}H_{50}NO_4$ requires 560.3746), *m/z* $[M\text{-}C_{10}H_{16}]^+$ 424.2498 ($C_{26}H_{34}NO_2$ requires 424.2482).

Preparation of 6,7-*O*,*O*-digeranyl-*N*-methylpapaveroline (13). To a stirred suspension of NaH (115

mg, 4.79 mmol) in DMF (15 mL) at rt under N_2 *N*-methylpapaveroline bromide[9] (**12**, 1 g, 2.66 mmol) was added portionwise followed by a catalytic amount of hydroquinone. The mixture was stirred for 30 min. Geranyl bromide (240 mg, 1.11 mmol) was added dropwise and the mixture was stirred for 10 h. Geranyl bromide (295 mg, 1.36 mmol) and NaH (70 mg, 2.92 mmol) were added dropwise and the mixture was stirred for a further 5 h. The reaction mixture was treated and separated by prep. HPLC as described in preparation of **10** and **11** to give 6,7-*O*,*O*-digeranyl-*N*-methylpapaveroline (**13**, 31 mg; yield 4.1%) as trifluoroacetate. **13**: ^{1}H NMR (500 MHz, CD_3OD) δ 8.32 (1H, d, J = 7.0 Hz, H-3), 8.08 (1H, d, J = 7.0 Hz, H-4), 7.70 (1H, s, H-8), 7.60 (1H, s, H-5), 6.71 (1H, d, J = 8.5 Hz, H-5′), 6.49 (1H, d, J = 1.5 Hz, H-2′), 6.36 (1H, dd, J = 8.5, 1.5 Hz, H-6′), 5.58 (1H, t, J = 6.5 Hz, H-2″), 5.39 (1H, t, J = 6.5 Hz, H-2‴), 5.09 (1H, m, H-6″), 5.02 (1H, m, H-6‴), 4.90 (2H, d, J = 6.5 Hz, H-1″), 4.83 (2H, s, H-9), 4.80 (2H, d, J = 6.5 Hz, H-1‴), 4.30 (3H, s, N-Me), 2.15 (H-5″), 2.13 (4H, s, H-4″), 2.05 (2H, m, H-5‴), 1.98 (2H, m, H-4‴), 1.83 (3H, s, 3″-Me), 1.70 (3H, s, 3‴-Me), 1.61 (3H, s, 8″), 1.59 (3H, s, 8‴), 1.58 (3H, s, 7″-Me), 1.55 (3H, s, 7‴-Me); ^{13}C NMR (125 MHz, CD_3OD) δ 158.86 (C-6), 156.93 (C-1), 154.18 (C-7), 147.50 (C-3′), 146.17 (C-4′), 144.48 (C-3″), 143.93 (C-3‴), 137.50 (C-4a), 136.61 (C-3), 132.81 (C-7″), 132.63 (C-7‴), 126.47 (C-1′), 125.62 (C-1a), 124.91 or 124.84 (C-6″or C-6‴), 123.58 (C-4), 120.39 (C-6′), 119.65 (C-2‴), 119.32 (C-2″), 117.14 (C-5′), 116.13 (C-2′), 108.15 (C-8), 108.08 (C-5), 67.71 (C-1″), 67.33 (C-1‴), 46.28 (N-Me), 40.63 (C-4″) , 40.46 (C-4‴), 34.96 (C-9), 27.30 or 27.29 (C-5″or C-5‴), 25.91 (C-8″), 25.87 (C-8‴), 17.82 or 17.81 (7″ or 7‴-Me), 16.88 or 16.84 (3″ or 3‴-Me); SIMS *m/z* $[M]^+$ 570; HRMS *m/z* $[M]^+$ 570.3592 ($C_{37}H_{48}NO_4$ requires 570.3583).

Preparations of (±)-(*cis*) and (±)-(*trans*)-*N*-geranyl-2,3,9,10-tetrademethyltetrahydropalmatinium salts (15 and 16). To a stirred suspension of NaH (126 mg, 5.25 mmol) in DMF (15 mL) at room temperature under N_2 (±)-2,3,9,10-tetrademethyltetrahydropalmatine hydrobromide[9] (**14**, 1 g, 2.63 mmol) was added by portion followed by a catalytic amount of hydroquinone. The mixture was stirred for 30 min. Geranyl bromide (571 mg, 2.38 mmol) was added dropwise and the mixture was stirred for 2.5 h. Geranyl bromide (220 mg, 1.01 mmol) and NaH (50 mg, 2.08 mmol) were added again and the mixture was further stirred for 2.5 h. After decomposition of excess NaH with MeOH (1 mL), the mixture was poured onto ice-water and extracted with Et_2O followed by $CHCl_3$. The Et_2O and $CHCl_3$ phases were separately dried and evaporated. The $CHCl_3$ extract was subjected to preparative HPLC [0.1M NH_4OAc (0.05% TFA)/MeOH (0.05% TFA) (A/B) initial 60/40, 0/100 (30 min)] to give (±)-(*cis*)-*N*-geranyl-2,3,9,10-tetrademethyltetrahydropalmatinium salt (**15**, 29.5 mg, yield 2.0%) and (±)-(*trans*)-*N*-geranyl-2,3,9,10-tetrademethyltetrahydropalmatinium salt (**16**, 137.6 mg, yield 9.5%) as trifluoroacetates. **15**: ^{1}H NMR (500 MHz, CD_3OD) δ 6.78 (1H, d, J = 8.0 Hz, H-11), 6.72 (1H, s, H-4), 6.66 (1H, s, H-1), 6.57

(1H, d, J = 8.0 Hz, H-12), 5.44 (1H, t, J = 8.0 Hz, H-2′), 5.08 (1H, brs, H-6′), 4.58 (3H, brs, H-8, H-13a), 4.08 (2H, m, H-1′), 3.81 (1H, m, H-6), 3.54 (1H, m, H-6), 3.28 (1H, m, H-13), 3.18 (2H, m, H-5), 3.12 (1H, m, H-13), 2.22 (2H, m, H-4′), 2.20 (2H, m, H-5′), 1.65 (3H, s, 3′-Me), 1.59 (3H, s, 8′), 1.58 (3H, s, 7′-Me); ^{13}C NMR (125 MHz, CD_3OD) δ 152.82 (C-3′), 147.66 (C-3), 146.25 (C-2), 144.85 (C-10), 143.60 (C-9), 133.47 (C-7′), 124.76 (C-1a), 124.12 (C-6′), 121.90 (C-12a), 120.93 (C-4a), 119.69 (C-12), 116.59 (C-4, C-11), 114.80 (C-8a), 114.52 (C-1), 111.74 (C-2′), 64.34 (C-13a), 59.93 (C-1′), 57.06 (C-8), 51.66 (C-6), 41.04 (C-4′), 34.89 (C-13), 26.97 (C-5′), 25.78 (C-8′), 23.75 (C-5), 17.79 (7′-Me), 16.94 (3′-Me);SIMS *m/z* $[M]^+$ 436; HRMS *m/z* $[M]^+$ 436.2486 ($C_{27}H_{34}NO_4$ requires 436.2482), *m/z* $[M\text{-}C_{10}H_{16}]^+$ 300.1230 ($C_{17}H_{18}NO_4$ requires 300.1235); **16**: ^{1}H NMR (500 MHz, CD_3OD) δ 6.85 (1H, d, J = 8.2 Hz, H-11), 6.84 (1H, s, H-1), 6.77 (1H, d, J = 8.2 Hz, H-12), 6.71 (1H, s, H-4), 5.44 (1H, dd, J = 9.5, 5.5 Hz, H-2′), 5.14 (1H, brs, H-6′), 5.07 (1H, dd, J = 12.5, 5.0 Hz, H-13a), 4.72 (1H, d, J = 15.5 Hz, H-8), 4.17 (1H, d, J = 15.5 Hz, H-8), 3.89 (1H, m, H-6), 3.86 (1H, m, H-1′), 3.83 (1H, m, H-13), 3.69 (1H, m, H-6), 3.61 (1H, m, H-1′), 3.54 (1H, m, H-6), 3.26 (1H, m, H-5), 3.20 (1H, dd, J = 12.5, 5.0 Hz, H-13), 3.05 (1H, brs, H-5), 2.20 (4H, m, H-4′, H-5′), 1.72 (3H, s, 8′), 1.66 (3H, s, 7′-Me), 1.33 (3H, s, 3′-Me); ^{13}C NMR (125 MHz, CD_3OD) δ 153.83 (C-3′), 147.37 (C-3), 146.63 (C-2), 144.95 (C-10), 143.66 (C-9), 133.45 (C-7′), 124.71 (C-6′), 122.77 (C-4a), 122.43 (C-12a), 122.06 (C-1a), 120.60 (C-12), 116.84 (C-11), 116.15 (C-4), 115.38 (C-8a), 113.45 (C-1), 111.42 (C-2′), 68.21 (C-13a), 58.43 (C-6), 57.62 (C-8), 46.96 (C-1′), 41.14 (C-4′), 29.60 (C-13), 27.10 (C-5′), 25.97 (C-8′), 24.48 (C-5), 17.79 (7′-Me), 16.0 (3′-Me);SIMS *m/z* $[M]^+$ 436; HRMS *m/z* $[M]^+$ 436.2484 ($C_{27}H_{34}NO_4$ requires 436.2482), *m/z* $[M\text{-}C_{10}H_{16}]^+$ 300.1230 ($C_{17}H_{18}NO_4$ requires 300.1235).

Preparation of (±)-(*trans*)-*N*-geranyl-2,3,10,11-tetrademethylpseudotetrahydropalmatinium salts (18). To a stirred suspension of NaH (130 mg, 5.42 mmol) in DMF (15 mL) at room temperature under N_2 (±)-2, 3, 10, 11-tetrademethylpseudotetrahydropalmatine hydrobromide[9] (**17**, 1 g, 2.63 mmol) was added by portions followed by a catalytic amount of hydroquinone. The mixture was stirred for 30 min. Geranyl bromide (580 mg, 2.67 mmol) was added dropwise and the mixture was stirred for 4.5 h. After decomposition of excess NaH with MeOH (1 mL), the mixture was poured onto ice-water. The precipitated crystals (703 mg) were subjected to prep. HPLC [0.1M NH_4OAc (0.05% TFA)/MeOH (0.05% TFA) (A/B) initial 60/40, 0/100 (30 min)] to give (±)-(*trans*)-*N*-geranyl-2,3,10,11-tetrademethylpseudotetrahydropalmatinium trifluoroacetate (**18**; 318.8 mg; yield 21.9%). **18**: ^{1}H NMR (500 MHz, CD_3OD) δ 6.83 (1H, s, H-1), 6.81 (1H, s, H-12), 6.70 (1H, s, H-4), 6.55 (1H, s, H-9), 5.44 (1H, m, H-2′), 5.12 (1H, brs, H-6′), 5.07 (1H, m, H-13a), 4.39 (1H, d, J = 15.0 Hz, H-8), 4.34 (1H, d, J = 15.0 Hz, H-8), 3.87 (1H, m, H-1′), 3.85 (1H, m, H-6), 3.78 (1H, m, H-13), 3.64 (1H, m, H-1′), 3.63 (1H, m, H-6), 3.25 (1H, m, H-5), 3.15 (1H, d, J = 13.0 Hz, H-13), 3.04 (1H, m, H-5), 2.20 (4H, m, H-4′, H-5′),