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Sampsoniones A–M, a Unique Family of Caged Polyprenylated Benzoylphloroglucinol Derivatives, from *Hypericum sampsonii*

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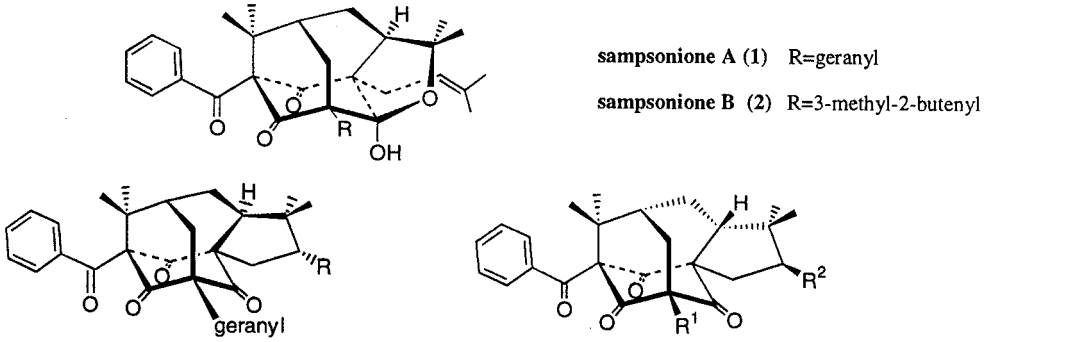
Abstract—Further thorough investigation of the ethanolic extract of the aerial parts of *Hypericum sampsonii* has yielded sampsoniones K–M which together with the earlier isolated sampsoniones A–J form a unique family of related polyprenylated benzoylphloroglucinol derivatives formed by complex cyclisations of prenyl groups. The structures of these cage compounds have been elucidated by extensive studies of various spectrometric techniques. The 13 metabolites, some of which are bioactive, are probably biosynthesized from a simple common benzoylphloroglucinol precursor.

Introduction

In recent years the widespread interest in the antidepressant activity of *Hypericum perforatum* (St. John's wort) has attracted much activity in investigating metabolites from the *Hypericum* genus, many of which are biologically active compounds with an acylphloroglucinol moiety.[1–11] *Hypericum sampsonii* is a Chinese herbal medicine used in the treatment of numerous disorders such as backache, burns, diarrhoea, snakebites and swellings.[12] *H. sampsonii* was previously reported to contain various xanthone derivatives.[13] We have recently carried out a detailed investigation on the metabolites of this plant and our preliminary communications[14–16] have reported the characterization of sampsoniones of A (**1**) and B (**2**), with the novel 5-oxatetracyclo-skeleton arising from cyclizations of two prenyl substituents, sampsoniones C–H (**3**–**8**), with an unusual carbotetracyclo-skeleton formed by complex cyclizations of three prenyl substituents and sampsoniones I–J (**9**–**10**) with an adamantyl skeleton. This paper reports the isolation of these novel metabolites as well as the isolation and characterisation of three more new compounds, sampsoniones K (**11**), L (**12**) and M (**13**), in addition to the known clusianone.[17] These 13 natural products form a unique



sampsonione A (1) R=geranyl

sampsonione B (2) R=3-methyl-2-butenyl

sampsonione C (3) R= 1-hydroxy-isopropyl

sampsonione D (4) R= 1-methylvinyl

sampsonione E (5) R=O (ketone)

sampsonione F (6) R^1=geranyl , R^2= 1-hydroxy-isopropyl

sampsonione G (7) R^1=3-methyl-2-butenyl , R^2=1-hydroxy-isopropyl

sampsonione H (8) R^1=geranyl , R^2=H

Figure 1.

Keywords: adamantanes; biogenesis; cage compounds; isoprenoids; NMR.

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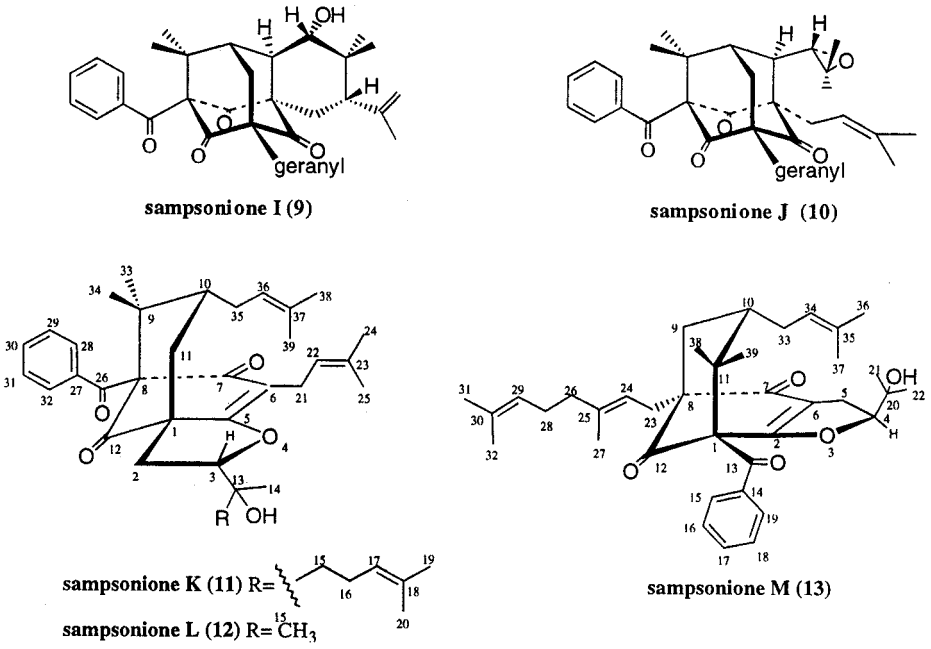

Figure 2.

family of structurally related, caged metabolites which are probably biosynthesized from a common benzoylphloroglucinol precursor.

Results and Discussion

The dried plant material was extracted with 95% EtOH and the concentrate was partitioned between CH_2Cl_2 and H_2O. The CH_2Cl_2 soluble portion was separated into nine fractions by silica gel column chromatography eluting with hexane–ethyl acetate. The third fraction eluted with hexane–ethyl acetate (10:1) was further chromatographed on silica gel, ODS, PTLC to give compounds **1**–**13** and clusianone (Figs. 1 and 2).

Sampsonione K (**11**) (127.5 mg, 0.00255%) was isolated as an optically active colourless oil, $[\alpha]_D^{31.2}=-5.60$ (*c*, 1.082, $CHCl_3$). HREIMS indicated a molecular formula of $C_{38}H_{50}O_5$ (*m*/*z* 586.36733). The UV spectrum exhibited maxima at 214 (3.97), 246 (3.91), 272 (3.52) nm. The IR spectrum showed strong bands for hydroxyl (3502 cm^{-1}) and carbonyl groups (1740, 1702, 1686 cm^{-1}). EIMS exhibited a base peak at *m*/*z* 105, arising from the diagnostic $[C_6H_5CO]^+$ fragment ion. The ^1H and ^{13}C NMR data of **11** (Table 1) indicated a structural variation from the caged sampsoniones A–J. The phloroglucinol part of the benzophenone backbone of K (**11**) has only one unconjugated carbonyl (205.5 ppm) and an enolised 1,3-dicarbonyl ether system (δ 193.4, 115.4 and 172.5). Based on 1D (^1H and ^{13}C) and 2D (^1H–^1H COSY, HMBC, HMQC and NOESY), the readily identifiable pendant residues on the main skeleton were (a) the gem-dimethyl group (C_{33} and C_{34}) correlated by HMBC to each other and to C_9; (b) a benzoyl group (C_{26}–C_{32}) on C_8; (c) a 3-methyl-2-butenyl side chain (C_{21}–C_{25}) on C_6; (d) a 3-methyl-2-butenyl side chain (C_{35}–C_{39}) on C_{10}; (e) a 4-methyl-3-pentenyl side chain (C_{15}–C_{20}) on C_{13} and (f) a methyl group (C_{14}) on C_{13}.

The structure of the tricyclic core of the molecule was determined by tracing the connectivities shown in the HMBC spectrum. Starting with the gem-dimethyl at C_9, cross-peaks were observed between protons of both methyl groups (δ 1.48 and 1.40) and (i) the quaternary carbon signal at δ 77.1 (C_8) which, from its typical highly deshielded position, had to be flanked by three carbonyl groups (shown as C_7, C_{12} and C_{26})[14–16,18]; (ii) the methine carbon at δ 47.6 (C_{10}). Moreover, one of the C_{11} methylene protons at δ 2.30 was correlated with the quaternary carbon signals at δ 58.7 (C_1), 172.5 (C_5), 49.1 (C_9), 205.5 (C_{12}) as well as the methine carbon signal at δ 47.6 (C_{10}), and the other C_{11} methylene proton at δ 2.17 was correlated with the quaternary carbon signals at δ 58.7 (C_1) and 172.5 (C_5) and the methine carbon signal at δ 47.6 (C_{10}). Therefore, carbons 1, 12, 8, 9, 10, 11 formed the six-membered carbon ring.

The ^1H NMR spectrum showed the presence of one methyl and one methylene adjacent to an oxygen-function (δ 1.35 and 2.17, 1.52), an oxygenated methine proton [δ 4.62 (1 H, dd, J=10.6, 5.7 Hz)], in addition to two methylene protons [δ 1.80 (1 H, dd, J=13.0, 5.7 Hz) and 2.72 (1 H, dd, J=13.1, 10.6 Hz)]. In the HMBC spectrum of **11**, the C_{14} methyl protons (δ 1.35) were correlated to the methine carbon with an oxygen-function (δ 89.7, C_3) and a quaternary carbon with an oxygen-function (δ 72.7, C_{13}). The latter carbon was further correlated to one of the C_2 methylene protons at δ 2.72. These results and the MS spectral data suggested that a possible partial structure of **11** was either A or B (Fig. 3). No reaction occurred on attempted acetylation of **11** with Ac_2O–pyridine at room temperature overnight, suggesting that the hydroxyl group was tertiary. NOE interactions were observed between the C_2 methylene proton at δ 2.72 and C_{14} methyl protons, C_{15} methylene protons, the results of which supported that A was preferable to B as a partial structure of **11**. There remained to be determined the orientation of a tetrahydrofuran ring. In the HMBC spectrum, the C_2 methylene protons showed cross-peaks to

Table 1. NMR data for sampsoniones K (**11**), L (**12**) and M (**13**)

Position	Sampsonione K (**11**)				Sampsonione M (**13**)				Sampsonione L (**12**)		
	H[a]	^{13}C[b]	DEPT	HMBC[c]	^{1}H[a]	^{13}C[b]	DEPT	HMBC[c]	^{1}H[a]	^{13}C[b]	DEPT
1		58.7				68.0				59.8	
2	proR 2.72 dd (13.1, 10.6) proS 1.80 dd (13.0, 5.7)	30.6	CH_2	1, 5, 11 1, 3, 11, 12, 13		173.7			proR 2.69 dd (13.6, 11.0) proS 1.82 dd (13.6, 6.2)	30.9	CH_2
3	4.62 dd (10.6, 5.7)	89.7	CH						4.62 dd (10.5, 5.7)	90.0	CH
4					4.02 dd (11.2, 10.2)	93.7	CH				
5		172.5			2.81 m	27.4	CH_2	2, 4, 6, 20		172.4	
6		115.4				117.9				115.4	
7		193.4				191.2				193.8	
8		77.1				63.0				77.1	
9		49.1			proR 2.10 dd (14.9, 1.8) proS 2.14 dd (14.9, 6.2)	39.8	CH_2	7, 23 8, 10, 12		49.2	
10	1.50 m	47.6	CH	9	1.42 m	48.5	CH		1.51 m	47.7	CH
11	proR 2.17 m proS 2.30 d (14.1)	37.0	CH_2	1, 5, 9, 10, 12, 35 1, 5, 10, 35		48.5			proR 2.17 m proS 2.32 d (14.0)	36.6	CH_2
12		205.5				207.3				205.4	
13		72.7				193.1				70.8	
14	1.35 s	23.0	CH_3	3, 13, 15		137.2			1.39 s	26.5	CH_3
15	2.17 m 1.52 m	36.6	CH_2	14	7.59 dd (8.6, 1.2)	127.8	CH	13, 17, 19	1.24 s	23.8	CH_3
16	2.13 m	21.8	CH_2	17, 18	7.30 t (8.5)	128.0	CH	14, 19			
17	5.12 t (7.5)	123.6	CH	19, 20	7.41 t (8.7)	131.2	CH	15, 19			
18		132.7			7.30 t (8.5)	128.0	CH	14, 16			
19	1.69 s	25.6	CH_3	17, 18, 20	7.59 dd (8.6, 1.2)	127.8	CH	13, 15, 17			
20	1.63 s	17.6	CH_3	17, 18, 19		70.5					
21	3.09 dd (14.4, 7.8) 3.03 dd (14.4, 8.0)	22.2	CH_2	5, 6, 7, 22, 23 5, 6, 7, 22, 23	1.14 s	24.8	CH_3	4, 20, 22	3.07 dd (14.5, 7.5) 2.99 dd (14.1, 8.3)	22.2	CH_2
22	5.05 t (8.1)	119.7	CH	24, 25	1.18 s	26.2	CH_3	4, 20, 21	5.05 t (7.1)	119.7	CH
23		132.7			2.62 dd (13.9, 6.9) 2.47 dd (13.9, 7.2)	29.6	CH_2	8, 12, 24, 25 7, 8, 9, 24, 25		132.5	
24	1.62 s	25.7	CH_3	22, 23, 25	4.85 t (6.5)	119.1	CH		1.70 s	25.7	CH_3
25	1.63 s	17.6	CH_3	22, 23, 24		138.2			1.64 s	17.6	CH_3
26		193.8			1.92 m	39.9	CH_2	24, 25, 27, 28		193.5	
27		136.8			1.70 s	16.4	CH_3	24, 25, 26		136.9	
28	7.45 dd (7.9, 0.8)	127.8	CH	26, 30, 32	2.04 m	26.7	CH_2	25, 26, 29, 30	7.45 d (7.0)	127.8	CH
29	7.20 t (7.8)	128.0	CH	27, 31	5.08 m	124.2	CH	26, 28	7.21 t (7.4)	128.0	CH
30	7.38 t (7.5)	131.8	CH	28, 32		131.2			7.38 t (7.0)	131.8	CH
31	7.20 t (7.8)	128.0	CH	27, 29	1.65 s	25.7	CH_3	29, 30, 32	7.21 t (7.4)	128.0	CH
32	7.45 dd (7.9, 0.8)	127.8	CH	26, 28, 30	1.58 s	17.6	CH_3	29, 30, 31	7.45 d (7.0)	127.8	CH
33	1.48 s	22.2	CH_3	8, 9, 10, 34	1.88 m 2.23 brd (14.6)	29.3	CH_2	10, 11, 34, 35 34, 35	1.48 s	22.2	CH_3
34	1.40 s	26.8	CH_3	8, 9, 10, 33	5.08 m	124.5	CH		1.41 s	26.8	CH_3
35	2.17 m	28.9	CH_2	10, 11, 36, 37		132.8			2.17 m	28.9	CH_2
36	4.88 t (6.9)	124.3	CH	38, 39	1.65 s	25.6	CH_3	34, 35, 37	4.89 t (7.0)	124.3	CH
37		132.3			1.53 s	17.9	CH_3	34, 35, 36		132.5	
38	1.69 s	25.6	CH_3	36, 37, 39	1.40 s	26.5	CH_3	1, 10, 11, 39	1.63 s	25.6	CH_3
39	1.55 s	17.6	CH_3	36, 37, 38	1.51 s	24.0	CH_3	1, 10, 11, 38	1.55 s	17.6	CH_3

[a] Recorded in $CDCl_3$ at 300 MHz.
[b] Recorded in $CDCl_3$ at 75 MHz.
[c] Carbons that correlate with the proton resonance.

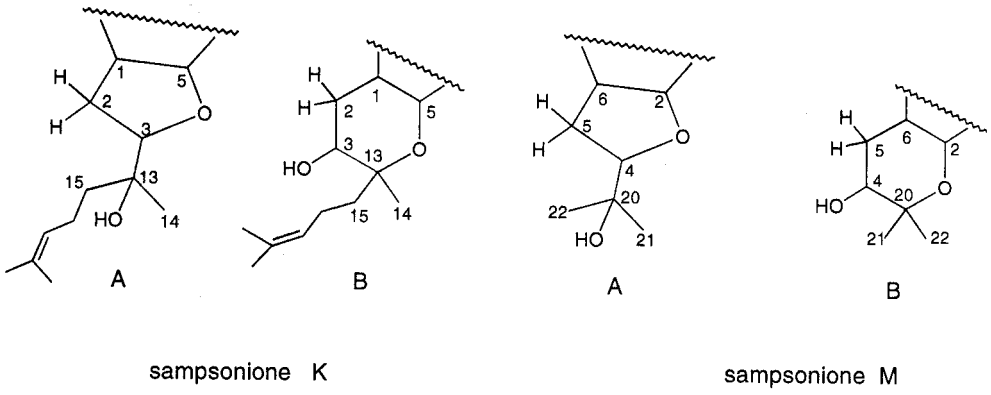

Figure 3. Two possible partial structures of sampsoniones K and M.

the quaternary carbons at δ 58.7 (C_1), 172.5 (C_5), 205.5 (C_{12}) and the methylene carbon at δ 37.0 ppm (C_{11}), supporting that the tetrahydrofuran ring was formed through the hydroxyl group at C_5.

Molecular models disclosed that, by its formation, the tricyclic system itself sets up the relative configurations at the chiral carbons at C_1 and C_8. The relative stereochemistry of the remaining chiral carbons at C_3 and C_{10} was determined by coupling constant analysis and NOE data (Fig. 4). The cross-peak between one of the C_{11} methylene protons at δ 2.30 ppm and the C_3 oxygen-bearing methine proton (δ 4.62) indicated that the C_3 proton has the β configuration as shown in **11**. The cyclohexanone ring adopts the chair conformation as the axial C_{11} proton showed NOE interactions with H-10 and C_{34} methyl protons and a coupling[19] to H_{10} which indicated that the isoprenyl group on C_{10} to be of axial orientation.

Further support for the axial orientation of the isoprenyl group at C_{10} comes from comparison of ^{1}H and ^{13}C NMR data with those of garcinol[20] and methylepigarcinol[21] (Fig. 5) which shows that when the C_{10} substituent is axial, the H-11 protons both resonate above 2.0 ppm and the axial H-11 shows one large and one small coupling. On the other hand, when the C_{10} substituent is equatorial, the axial H-11 proton resonates at ca. 1.5 and has large couplings.[20,21] The ^{13}C NMR spectrum also provides a method for establishing the stereochemistry at C_{10}. The axial methyl attached to C_9 is much more shielded when the C_{10} substituent is equatorial (16.4 in the case of methylepigarcinol as opposed to 27.3 in the case of garcinol) due to the γ-*gauche* interaction.[20] The corresponding relevant ^{1}H and ^{13}C NMR data for sampsonione K is similar to garcinol and hence it can be assigned the structure (**11**).

The second minor constituent, sampsonione L (**12**) (2.8 mg, 0.000056%), HREIMS $[M]^+$ 518.30597, calcd for $C_{33}H_{42}O_5$, 518.30322, had UV and IR spectral features similar to those of **11**. A comparison of the ^{1}H and ^{13}C NMR data of **12** (Table 1) with those of **11** revealed that the only difference was in the side chain at C_{13}, the 4-methyl-3-pentenyl group in **11** being replaced by a methyl function in **12**. Its structure was confirmed by its ^{1}H–^{1}H COSY, HMQC, HMBC and NOESY spectra.

The third minor constituent, sampsonione M (**13**) (2.2 mg, 0.000044%), was obtained as an optically active colourless oil, $[\alpha]_D^{31.2}=+54.77$ (*c*, 0.044, $CHCl_3$), with the following spectral characteristics: IR (film) ν_{max} 3452, 1730, 1699, 1653, 1622 cm^{-1}; UV (MeOH) λ_{max} (log ϵ) 212 (3.97), 250 (3.96), 280 (3.82); ^{1}H and ^{13}C NMR, Table 1.

HREIMS indicated a molecular formula of $C_{38}H_{50}O_5$ (*m*/*z* 586.36787). NMR data (Table 1) indicated it is similar to **11** and **12**. The phloroglucinol part of the benzophenone backbone has one unconjugated carbonyl (δ 207.3 ppm) and an enolised 1,3-dicarbonyl ether system (δ 191.2, 117.9 and

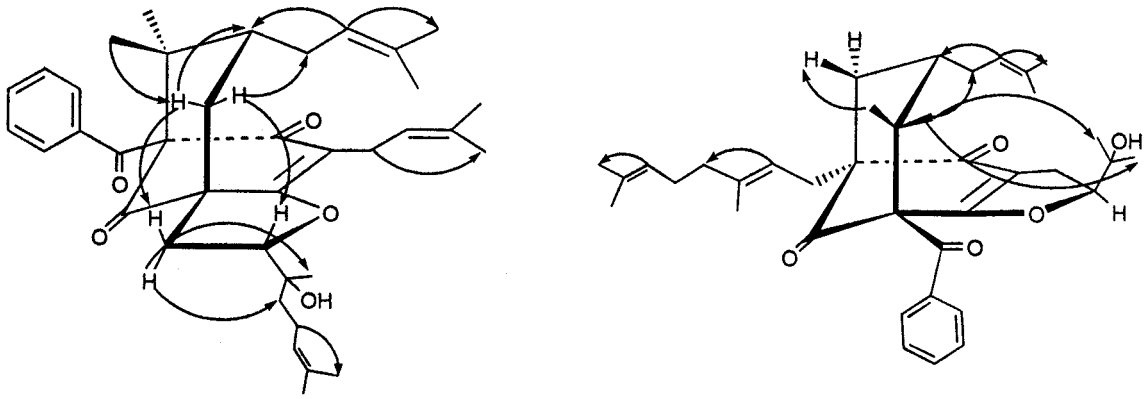

Figure 4. Selected NOESY cross-peaks of sampsonione K (**11**) and M (**13**).

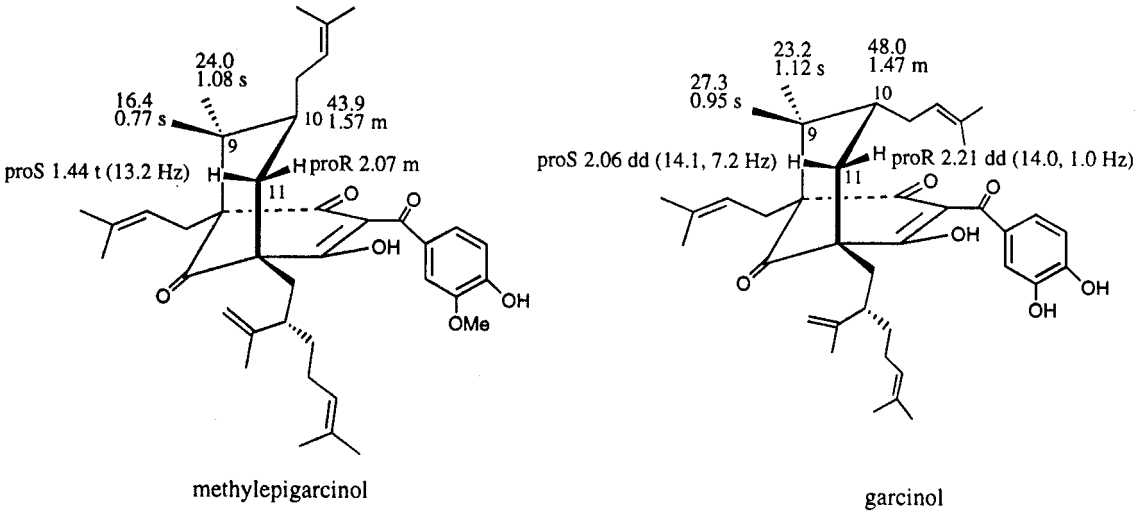

Figure 5. Selected ^{1}H and ^{13}C chemical shifts of methylepigarcinol and garcinol.

173.7). Based on 1D (^{1}H and ^{13}C) and 2D (^{1}H–^{1}H COSY, HMBC, HMQC and NOESY), the readily identifiable pendant residues on the main skeleton were (a) the gem-dimethyl group (C_{38} and C_{39}) correlated by HMBC to each other and on C_{11}; (b) the gem-dimethyl group (C_{21} and C_{22}) correlated by HMBC to each other and to C_{20}; (c) a benzoyl group (C_{13}–C_{19}) on C_1; (d) a 3-methyl-2-butenyl side chain (C_{33}–C_{37}) on C_{10} and (e) a geranyl side chain (C_{23}–C_{32}) on C_8.

The structure of the tricyclic core of the molecule was determined by tracing the connectivities shown in the HMBC spectrum. Starting with the gem-dimethyl at C_{11}, cross-peaks were observed between protons of both methyl groups (δ 1.40 and 1.51) and: (i) the quaternary carbon signal at δ 68.0 (C_1) which, from its deshielded position, had to be flanked by two carbonyl groups (C_{12} and C_{13}); (ii) the methine carbon at δ 48.5 (C_{10}). Moreover, one of the C_9 methylene protons at δ 2.14 was correlated with the quaternary carbon signals at δ 191.2 (C_7) and the other C_9 methylene proton at δ 2.10 was correlated with the quaternary carbon signals at δ 63.0 (C_8) and 207.3 (C_{12}) and the methine carbon signal at δ 48.5 (C_{10}). Therefore, carbons 1, 12, 8, 9, 10 and 11 formed the six-membered cyclohexanone ring.

The ^{1}H NMR spectrum showed the presence of two methyls adjacent to an oxygen-function (δ 1.14 and 1.18 ppm), an oxygenated methine proton [δ 4.02 (1 H, dd, J=11.2, 10.2 Hz)], in addition to two methylene protons [δ 2.81 (2H, M)]. In the HMBC spectrum of **13**, the methyl protons (δ 1.14) were correlated to the methine carbon with an oxygen-function (δ 93.7) and a quaternary carbon with an oxygen-function (δ 70.5). The latter carbon was further correlated to the methylene protons at δ 2.81. These results and the MS spectral data suggested that **13** has a partial structure of either A or B (Fig. 3). The failure of **13** to undergo acetylation, indicating that the hydroxyl was tertiary, and the observation of NOE interactions between the C_5 methylene protons at δ 2.81 and C_{21}, C_{22} methyl protons supported that partial structure A was preferable to B. There remained to be determined the orientation of the dihydrofuran ring. In the HMBC spectrum, the C_5 methylene protons showed cross-peaks to the quaternary carbons at δ 73.7 (C_2), 117.9 (C_6), and the methine carbon at δ 93.7 (C_4), supporting that the dihydrofuran ring was formed through the hydroxyl group at C_2.

Assignment of the relative stereochemistry of **13** was based principally on coupling constant analysis and NOE data (Fig. 4). The tricyclic ring system in **13** required that the geranyl and the benzoyl groups on C_1 and C_8 to be equatorial. The axial C_9 proton showed a small coupling to H-10 and NOE interactions with H-10 and C_{38} methyl protons, which supported that the isoprenyl group on C_{10} to be of axial orientation. Further support of this assignment comes from comparison of its relevant ^{1}H and ^{13}C NMR chemical shifts which were more similar to those of garcinol[20] than of methylepigarcinol[21] (see Fig. 5). Cross-peaks between the C_{21}, C_{22} methyls (δ 1.14 and 1.18) and the C_{39} methyl (δ 1.51) established the C_4 proton to be of α configuration as shown in **13**.

Sampsoniones A–M form a unique family of polyprenylated benzoylphloroglucinol derivatives, many of which have unprecedented, caged structures. They are presumably biosynthesized from the biogenetically acceptable 2,4,6-trihydroxybenzophenone **14** (Fig. 6). C-alkylation of 14 by geranyl pyrophosphate and 3,3-dimethylallyl pyrophosphate yields the intermediate **15**, which in turn could react with one more prenyl group as indicated leading to a bicyclo[3.3.1]nonanetrione **16**, which is the common precursor of sampsoniones A–M. **16** subsequently epoxidizes and intramolecularly cyclizes to form a tricyclo[4.3.1.1]undecanetrione **17**. In this ring system the carbonyl carbon on the bridge and the hydroxyl group are close to each other, thereby leading to a favorable relationship for hemiketal formation to the novel rigid 5-oxatetracyclo[7.3.1.$0^{3,7}$.$0^{4,11}$]tridecane-2,12-dione skeleton of sampsonione A (**1**) and sampsonione B (**2**). Epoxidation and intramolecular cyclization of **16** establish the tetracyclo[7.3.1.$1^{3,11}$.$0^{3,7}$]tetradecane-2,12,14-trione skeleton of sampsoniones C (3), F (**6**) and G (**7**), and these subsequently dehydrate to form sampsonione D (**4**) and **4a**. The

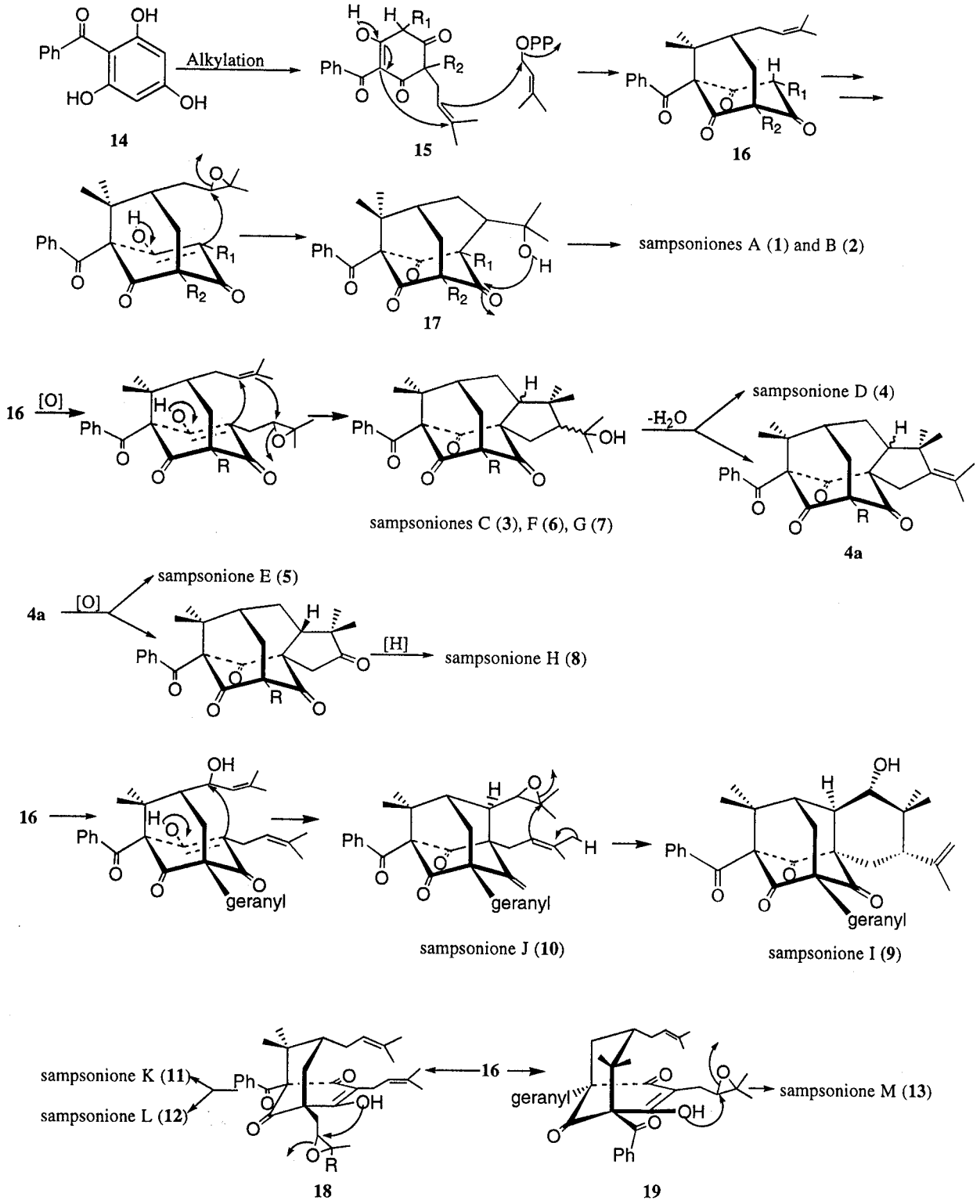

Figure 6. Possible biosynthesis pathway of sampsoniones A–M.

intermediate **4a** undergoes oxidation and reduction to yield sampsoniones E (**5**) and H (**8**). Allylic hydroxylation and intramolecular cyclization of **16** give the adamantyl backbone intermediate, which subsequently epoxidizes to form sampsonione J (**10**). **10** undergoes further intramolecular cyclization to yield sampsonione I (**9**). Epoxidation of **16** followed by enolisation yields intermediates **18** and **19**, the former undergoes intramolecular cyclizations to form sampsoniones K (**11**) and M (**12**), while the latter undergoes intramolecular cyclization to give sampsonione M (**13**).

Some support for the above biosynthetic proposal was provided by an acid-catalyzed biomimetic synthesis of sampsonione I (**9**) from the intramolecular cyclization of

sampsonione J (**10**) in an aprotic solvent. Treatment of **10** with *p*-toluenesulfonic acid in toluene at room temperature afforded a product in 90% yield which was identical to sampsonione I (**9**) in its ^{1}H and ^{13}C NMR spectra and optical rotation.

Most of the polyprenylated phloroglucinol derivatives isolated from *Hypericum* species contain an acyl group and it is interesting to note that *H. sampsonii* is the first found to contain so many complex polyprenylated phoroglucinol derivatives with an aroyl group which is more characteristic of metabolites obtained from other Guttiferous plants from the genera *Garcinia* and *Clusia*.

Several of the present compounds were evaluated for their cytotoxic effect against P388 cancer cell line. Sampsoniones A and I were found to be active 13 $\mu g\,mL^{-1}$ and 6.9 $\mu g\,mL^{-1}$, respectively.

Experimental

General

EIMS were run on a Micromass VG 7035 mass spectrometer at 70 eV. NMR spectra were determined on a Bruker ACF 300 [300 MHz (^{1}H) and 75 MHz (^{13}C)] and AMX 500 [500 MHz (^{1}H) and 125 MHz (^{13}C)] instruments using $CDCl_3$ solutions with TMS as an internal standard. IR spectra were recorded on a Bio-Rad FTIR spectrophotometer and UV spectra were recorded on a Hewlett Packard 8452A diode array spectrophotometer.

Plant material

The whole plant of *Hypericum sampsonii* was collected from Jinhua, Zhejiang Province, People's Republic of China in August 1997. It was identified by Dr X. Q. Ma and a voucher specimen (No. 97006) has been deposited at the herbarium of the Shanghai Institute of Materia Medica, Chinese Academy of Sciences, Shanghai, People's Republic of China.

Extraction and isolation

The whole air-dried ground plants (5 kg) were extracted at room temperature with 95% EtOH for seven days, the extract was concentrated in vacuo and the residue partitioned between CH_2Cl_2 and H_2O. The CH_2Cl_2-soluble portion (230 g) was then separated into nine fractions by silica gel cc, eluted with different proportions of hexane–ethyl acetate (1:0, 20:1, 10:1, 5:1, 2:1, 1:1, 1:2, 1:5, 0:1). The third fraction (70 g) was rechromatographed on silica gel and eluted with hexane–chloroform–acetone (6:3:0.5) to give four fractions I–IV. Fraction I was purified by prep. TLC, developed with hexane–ethyl acetate (10:1) to give clusianone (50.0 mg) and a mixture (15 mg). In the final purification of the mixture by ODS using acetone–water (9:1), **4** (6.6 mg) and **8** (6.6 mg) were isolated. Fraction II was subjected to VLC on ODS, eluted with acetone– water (10:1) to give **1** (120.0 mg) and **10** (58.4 mg). Fraction III was subjected to VLC on ODS, eluted with acetone–water (4:1) to give **7** (1.3 mg) and mixtures A and B. Final purification of the mixture A was achieved by prep. TLC, developed with hexane–ethyl acetate (5:1) to yield **2** (1.3 mg), **3** (10.5 mg), **5** (7.8 mg), **6** (54.8 mg), and **11** (127.5 mg). The mixture B was repurified on prep. TLC, developed with hexane–ethyl acetate–methanol (10:1:0.25) to give **9** (6.4 mg) and **13** (2.2 mg). Fraction IV was subjected to VLC on ODS, eluted with acetone–water (4:1) and finally purified on prep. TLC, developed with hexane–ethyl acetate (5:1) to yield **12** (2.8 mg).

Sampsonione K (11). Oil; $[\alpha]_D^{31.2}=-5.60$ (*c*, 1.082, $CHCl_3$); HREIMS: *m/z* 586.36733, $C_{38}H_{50}O_5$ requires 586.36584; IR (film) ν_{max} 3502, 1740, 1702, 1686 cm^{-1}; UV (MeOH) λ_{max} (log ϵ) 214 (3.97), 246 (3.91), 272 (3.52); EIMS *m/z* 586 $[M]^+$, 571, 517, 449, 417, 309, 231, 105, 77, 69, 43; ^{1}H and ^{13}C NMR, Table 1.

Sampsonione L (12). Oil; $[\alpha]_D^{31.2}=+55.00$ (*c*, 0.056, $CHCl_3$); HREIMS: *m/z* 518.30597, $C_{38}H_{50}O_5$ requires 518.30322; IR (film) ν_{max} 3468, 1730, 1699, 1630, 1452 cm^{-1}; UV (MeOH) λ_{max} (log ϵ) 214 (3.97), 242 (4.08), 274 (4.03); EIMS *m/z* 518 $[M]^+$, 503, 449, 417, 381, 105, 77; ^{1}H and ^{13}C NMR, Table 1.

Sampsonione M (13). Oil; $[\alpha]_D^{31.2}=+54.77$ (*c*, 0.044, $CHCl_3$); HREIMS: *m/z* 586.36787, $C_{38}H_{50}O_5$ requires 586.36584; IR (film) ν_{max} 3452, 1730, 1699, 1653 cm^{-1}; UV (MeOH) λ_{max} (log ϵ) 212 (3.97), 250 (3.96), 280 (3.82); EIMS *m/z* 586 $[M]^+$, 568, 517, 435, 255, 105, 77, 43; ^{1}H and ^{13}C NMR, Table 1.

Acid-catalysed transformation of sampsonione J (10). Sampsonione J (**10**) (50 mg) was dissolved in toluene (10 mL) and *p*-toluenesulfonic acid (120 mg) was added. The mixture was stirred at room temperature and monitored by TLC. After 6 h the reaction was complete and the solvent removed in vacuo to give a purple oil (100 mg). VLC separation of the crude product on RP-18 silica gel using acetone–water (4:1), provided sampsonione I (45.3 mg).

Bioassay

The P388 (mouse lymphocytic leukemia) cell line was used. Cell survival was evaluated by using MTT–tetraazolium assay as described previously.[22]

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