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THE GUTTIFERONES, HIV-INHIBITORY BENZOPHENONES FROM *Symphonia globulifera, Garcinia livingstonei, Garcinia ovalifolia* and *Clusia rosea*[1]

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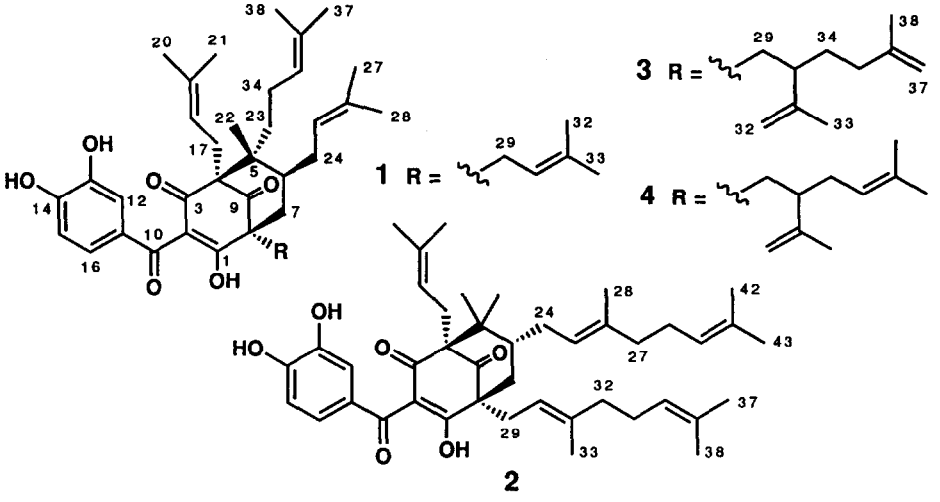
Summary: Extracts from species of the tropical plant genera *Symphonia*, *Garcinia* and *Clusia* (Guttiferae) have yielded a series of new polyisoprenylated benzophenone derivatives named guttiferones A-E (**1-5**). Structural assignments were based on detailed spectral analyses. These compounds inhibit the cytopathic effects of *in vitro* HIV infection.

Recently, we traced the anti human immunodeficiency virus (HIV) activity[3,4] detected in an extract of *Calophyllum lanigerum* (Guttiferae) to a series of coumarins.[5] Organic extracts from four other members of the Guttiferae, *Symphonia globulifera*, *Garcinia livingstonei*, *G. ovalifolia* and *Clusia rosea*, were also found active in our primary screen. The only chemistry previously reported for *S. globulifera* was the occurrence of various xanthone derivatives.[6,7] Extensive phytochemical studies have shown *Garcinia* to be a rich source of secondary metabolites including xanthones,[8] flavanoids,[9,10,11] benzophenones,[12,13,14] lactones[14] and phenolic acids.[15] Bioassay-guided fractionation of the *S. globulifera* extract tracked the HIV-inhibitory activity to a series of new prenylated benzophenone derivatives which we have named guttiferones A-D (**1-4**). Similar fractionation of the *G. livingstonei* extract provided guttiferone A (**1**) as the primary anti-HIV active constituent, while two benzophenone compounds, guttiferone E (**5**) and the known metabolite isoxanthochymol (**6**),[16,17,18] were obtained from *G. ovalifolia*. The *C. rosea* extracts were found to contain guttiferone E (**5**) and xanthochymol (**7**).

The CH_2Cl_2/MeOH extract of ground *S. globulifera* roots showed reproducible anti-HIV activity in the primary screen. Partitioning the crude extract between EtOAc and H_2O concentrated the activity in the EtOAc layer. The EtOAc solubles were sequentially chromatographed on diol and C_{18} bonded phases to provide active fractions containing a series of anti-HIV benzophenone derivatives. Final separation by C_{18} HPLC gave pure guttiferones A (**1**) and B (**2**), while guttiferones C (**3**) and D (**4**) were obtained as an inseparable mixture. Repeated attempts to separate these two compounds on diol, cyano, phenyl, C_8, C_{18}, polybutadiene, styrene/divinyl benzene and β-cyclodextrin supports were unsuccessful.

Guttiferone A (**1**) was isolated as a yellow oil, $[\alpha]_D$ +34°; HREIMS indicated a molecular formula of $C_{38}H_{50}O_6$ (*m/z* 602.3613). UV absorptions at 280 (ε 27,300) and 228 (ε 22,500) nm revealed a chromophore with extended conjugation. The IR spectrum showed strong bands for hydroxyl (3350 cm^{-1}) and both nonconjugated (1732 cm^{-1}) and conjugated (1644 cm^{-1}) carbonyl groups. Four vinyl protons, eight vinylic methyl groups and eight

allylic protons apparent in the ^{1}H NMR spectrum (Table 1) of **1** indicated the presence of four isopent-2-enyl groups. An aromatic AMX system was evident from proton resonances at δ 6.69 d (J = 8.3 Hz), 6.97 dd (8.3, 2.2), and 7.16 d (2.2). Characteristic ^{13}C NMR resonances, including those for substituted aromatic carbons at δ 129.3, 146.1 and 152.5 and a conjugated carbonyl at δ 195.5, were indicative of a 3,4-dihydroxybenzoyl group. Resonances for a six-membered ring consisting of a nonconjugated ketone (δ 209.5) flanked by two quaternary carbons (δ 62.1, 68.8) and an enolized 1,3-diketone (δ 117.7, 195.1, 195.4) were also observed. These data suggested that guttiferone A (**1**) was a polyisoprenyl benzophenone derivative related to xanthochymol (**7**).[17,18,19] Support for this assignment was provided by ^{13}C NMR signals for quaternary (δ 51.9), methine (δ 41.1) and methylene (δ 40.1) carbons which are part of the bicyclo[3.3.1]nonane ring system. As indicated by the ^{1}H NMR data, compound **1** possessed only one aliphatic methyl singlet (δ 1.24) and lacked any terminal olefins. It clearly differed from xanthochymol (**7**) in the nature and substitution pattern of the isoprenoid groups. Multiple bond heteronuclear correlation (HMBC) data unambiguously established that isopentenyl groups were substituted at C4, C6 and C8. The C23 methylene protons showed vicinal couplings to the C34 allylic protons and long range heteronuclear correlations to C5, C6, C22 and C35; thus the fourth isopentenyl moiety was attached at C23. Guttiferone A (**1**) is the first compound in this class with a C23 isopentenyl substituent.



Assignment of the relative sterochemistry of **1** was based principally on coupling constant analysis and nOe data, while assignment of the methylene protons at C7, C17, C23, C24 and C29 as proR or proS relied on nOe results and the dihedral angular dependence of three bond C-H correlations.[20] The bicyclic ring system in **1** required that the isopentenyl groups on C4 and C8 be equatorial. The C7 proS (axial) proton showed a 7.1 Hz coupling to H6 and nOe interactions with H6 and H23 proR. This established that C23 and C24 had axial orientations. NOe interactions between the C22 methyl protons and protons on C17 and C24 were consistent with these assignments.

Both the ^{1}H and ^{13}C NMR spectra of **1** obtained in $CDCl_3$ showed the presence of two constituents in *ca* 3:1 ratio. When the solvent was changed to CD_3OD/0.1% TFA, only a single set of resonances was observed. Signal broadening was removed on addition of TFA. This multiplicity of peaks in $CDCl_3$ solution apparently

Table I. NMR Data for Guttiferone A (1) and Guttiferone B (2)

	Guttiferone A (1)				Guttiferone B (2)			
Position	C[a]	H[b]	HMBC[c]	NOE[d]	C[a]	H[b]	HMBC[c]	NOE[d]
1	195.4				192.0			
2	117.7				119.1			
3	195.1				192.0			
4	68.8				69.7			
5	51.9				48.1			
6	41.1	1.83, m	4, 5, 7, 8, 22, 24, 25		44.2	1.60, m		
7	40.1	1.97 proS, dd (14.3, 7.1) 2.09 proR, dd (14.3, 1.2)	1, 6, 24, 29 1, 5, 6, 9, 24	6, 7R, 23R, 29R	43.1	1.47 proS, dd (13.2, 10.4) 2.02 proR, m	1, 8, 29	7R, 23, 24RS, 29
8	62.1				63.5			
9	209.5				208.8			
10	195.5				196.4			
11	129.3				129.9			
12	117.3	7.16, d (2.2)	10, 13, 14, 16		117.2	7.21, d (2.1)	10, 13, 14, 16	
13	146.1				146.2			
14	152.5				152.4			
15	115.1	6.69, d (8.3)	11, 13, 14		115.0	6.65, d (8.3)	11, 13, 14	
16	125.0	6.97, dd (8.4, 2.2)	10, 12, 14		125.1[e]	6.96, dd (8.3, 2.1)	11, 12, 14	
17	26.6	2.62 proS, dd (13.8, 4.9) 2.66 proR, dd (13.8, 8.8)	3, 4, 5, 9, 18, 19 4, 9, 18, 19	21, 22, 23 21, 22, 23	26.1	2.60 proS, ddd (13.8, 5.0, 0.9) 2.70 proR, dd (13.8, 8.5)	4, 5, 9, 18, 19	17R, 23 17S, 22
18	120.7	4.92, m	17, 20, 21	12, 16, 17S, 20	121.1	4.90, m	20, 21	
19	135.6				135.3			
20	26.0	1.63, s			26.3	1.63, s	18, 19, 21	
21	18.2	1.67, s			18.3	1.68, s	18, 19, 20	
22	19.7	1.24, s	5, 6, 23	17S, 23S, 24R, 34	23.8	1.14, s	4, 5, 6, 23	6, 17R, 23, 24S
23	36.9	1.20 proR, ddd (14.2, 10.4, 6.5) 1.39 proS, ddd (14.4, 10.1, 6.2)	5, 22, 35 5, 6, 35	7S, 23S, 34, 35 17R, 22, 23R, 34, 35	16.5	0.80, s	4, 5, 6, 22	7S, 17S, 22, 24RS
24	29.8	2.06 proS, m 2.09 proR, m	5, 6, 7, 25, 26		29.2	1.74 proR, ddd (13.9, 9.4, 9.4) 2.14 proS, ddd (13.9, 7.5, 0.9)	6, 25, 26 6, 7, 25, 26	23, 24S 6, 22, 24R, 25
25	125.4	4.88, ddq (7.0, 7.0, 1.3)	24, 27, 28		123.9	4.99, ddq (8.6, 7.3, 1.2)	24, 27, 28	24RS, 27
26	133.7				137.8			
27	26.3	1.64, d (1.3)	25, 26, 28		41.0[f]	1.96 (2H), m		
28	18.2	1.47, s	25, 26, 27		16.3	1.55, s	25, 26, 27	
29	32.0	2.44 proS, dd (14.3, 6.6) 2.50 proR, dd (14.3, 8.0)	1, 7, 9, 30, 31 1, 7, 9, 30, 31		31.2	2.50 (2H), m	8, 30, 31	7, 30, 33
30	120.8	5.20, ddq (8.0, 6.6, 1.2)	29, 32, 33	12, 16, 29RS, 32	120.6	5.16, ddq (7.1, 7.1, 1.3)	29, 32, 33	29, 32
31	135.7				138.9			
32	26.3	1.71, d (1.2)			40.8[f]	1.96 (2H), m		
33	18.3	1.67, s			16.9	1.67, d (1.3)	30, 31, 32	
34	23.7	1.87 (2H), m	23, 35, 36		27.7[g]	1.98, m 2.01, m		
35	125.1	5.07, ddq (7.3, 7.2, 1.2)	23, 34, 37, 38	34, 37	125.1[e]	5.05, m	34, 37, 38	34, 37
36	132.8				132.2			
37	25.9	1.67, s	35, 36, 38		26.0[h]	1.66, s	35, 36	
38	17.7	1.58, d (1.2)	35, 36, 37		17.8	1.58, s	35, 36	
39					27.5[g]	1.98, m 2.01, m		
40					125.0[e]	2.01, m	39, 42, 43	39, 42
41					132.2			
42					25.9[h]	1.58, s	40, 41	
43					17.8	1.53, d (1.2)	40, 41	

[a]Recorded in CD_3OD with 0.1% TFA at 125 MHz. [b]Recorded in CD_3OD with 0.1% TFA at 500 MHz. [c]Carbons that correlate with the proton resonance. [d]Resonance in the proton column irradiated. [e,f,g,h]May be interchanged within a column.

results from a keto-enol equilibrium in $CDCl_3$; in CD_3OD/TFA the rate of keto-enol tautomerism is enhanced to the point where only a time-average is observed. Although the NMR spectra of numerous polyprenylated benzophenone derivatives obtained in $CDCl_3$ have been reported, the presence of tautomers as evidenced by two sets of NMR signals has only been mentioned once.[21]

Guttiferone B (**2**), $C_{43}H_{58}O_6$ (*m/z* 670.4184), had UV and IR spectral features similar to those of **1** and showed characteristic ^1H and ^{13}C NMR resonances for a 3,4-dihydroxybenzoyl group and an enolized triketo bicyclo[3.3.1]nonane system. NMR analysis revealed five trisubstituted olefins, eight olefinic methyl groups and fourteen allylic methylene protons, which suggested the presence of one isopentenyl and two geranyl side chains. HMBC correlations required the placement of an isopentenyl group at C4. *Gem*-dimethyl substituents at C5 were indicated by HMBC correlations between the C22, C23 methyls and C4, C5 and C6. The C29 protons showed heteronuclear correlations to C8 and nOe interactions with the C33 methyl, while the C30 olefinic proton correlated with C32 and showed nOe enhancements with the C32 methylene protons. This allowed assignment of a geranyl moiety at C8 and revealed that the geometry of the C30-C31 olefin was *E*. The C25 olefinic proton had an HMBC correlation to C27 and nOe interactions with the C27 methylene protons. These data, together with HMBC correlations observed between the C24 protons and C6 and C7, established that the remaining geranyl group had an *E* olefin and was located at C6. A 10.4 Hz coupling between H6 and H7 (proS) required these protons to be diaxial and thus the geranyl group substituted at C6 was equatorial. All nOe interactions observed between the C24 protons, the C7 protons and the *gem*-dimethyl groups at C5 were fully consistent with this assignment. Guttiferone B (**2**) is the first member of this class of benzophenone derivatives in which the C4, C6 and C8 side chains all have *cis*-equatorial orientations.

Guttiferones C (**3**) and D (**4**) occured in *ca* 2:1 ratio and were characterized as an inseparable mixture. The mixture gave a single molecular ion at *m/z* 670.4274, corresponding to a molecular formula of $C_{43}H_{58}O_6$, and revealing that the two compounds were isomeric. The IR, UV, ^1H and ^{13}C NMR spectral characteristics of the mixture clearly indicated that compounds **3** and **4** were related to guttiferone A (**1**). Detailed NMR analysis of the mixture allowed assignment of the ^1H and ^{13}C resonances in both compounds (Table 2). HMBC correlations were particularly valuable in assigning isopentenyl substituents at C4, C6 and C23. The absence of the characteristically high-field protons for one of the *gem*-dimethyl groups substituted at C5 supported the placement of an isopropylidine moiety on C23. Both components of the mixture provided ^{13}C NMR resonances that were almost identical to those observed for guttiferone A (**1**), with the exception of those signals associated with the side chain attached at C8. Two sets of terminal methylene protons and two vinyl methyl groups were clearly assigned to the ten carbon sidechain in **3**, while the sidechain in **4** showed one set of terminal methylene protons and one trisubstututed olefin. These differences could best be explained if guttiferone C (**3**) had a side chain at C8 similar to compound **5** and guttiferone D (**4**) had a side chain similar to that of xanthochymol (**7**). HMBC correlations and close correspondence between the side chain ^{13}C resonances of **3** and **5** and the respective resonances of **4** and **7**[17,18,19] supported these assignments.

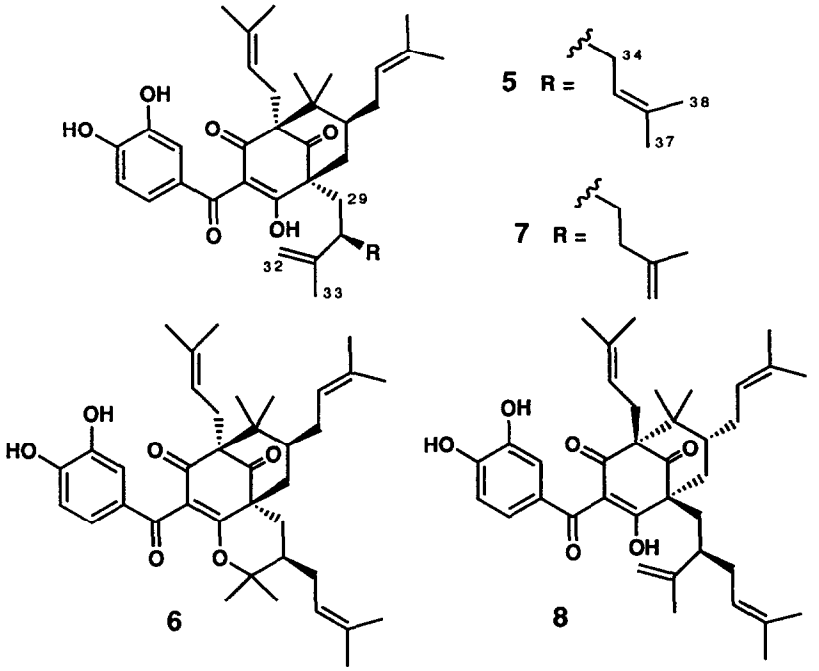
Bioassay guided fractionation of the HIV inhibitory extracts of the fruit of Garcinia livingstonei led to the isolation of guttiferone A (**1**) as the principal active constituent, identical in all respects to guttiferone A isolated from S. globulifera.

Table II NMR Data for Guttiferone C (3) and Guttiferone D (4)

	Guttiferone C (3)				Guttiferone D (4)			
Position	C[a]	H[b]	HMBC[c]	NOE[d]	C[a]	H[b]	HMBC[c]	NOE[d]
1	e				e			
2	117.9				117.8			
3	e				e			
4	70.5				70.5			
5	53.0				53.0			
6	41.2	1.80	4, 5, 7, 8, 24, 25		41.2	1.80	4, 5, 7, 8, 24, 25	
7	43.6	1.90 proS	1, 6, 24		43.6	1.90 proS	1, 6,24	
		2..22 proR	1, 5, 6, 8, 9, 24			2.22 proR	1, 5, 6, 8, 9, 24	
8	60.2				60.2			
9	210.21				210.16			
10	e				e			
11	129.4				129.3			
12	117.3	7.18	14, 16		117.2	7.18	14, 16	
13	146.2				146.2			
14	152.4				152.4			
15	115.0	6.69	11, 13, 14		115.0	6.68	11, 13, 14	
16	125.1	6.98	10, 12, 14		125.2	6.96	10, 12, 14	
17	26.6	2.62 (2H)	4, 9, 18, 19		26.7	2.62 (2H)	4, 9, 18, 19	
18	121.26	5.02	17, 20, 21	12, 16, 17, 20	121.29	5.02	17, 20, 21	12, 16, 17, 20
19	135.8				135.8			
20	26.44	1.71	18, 19, 21	12, 15, 16, 18	26.46	1.71	18, 19, 21	12, 15, 16, 18
21	18.4	1.56			18.4	1.56		
22	19.4	1.16	4, 5, 6, 23		19.4	1.16	4, 5, 6, 23	
23	36.42	1.22 proR	5, 22, 39	23S, 39, 40	36.39	1.22 proR	5, 22, 39	23S, 39, 40
		1.36 proS	5, 6, 39, 40	17, 23SR 39, 40		1.36 proS	5, 6, 39, 40	17, 23R, 39, 40
24	29.98	2.02 (2H)	6, 7, 25, 26		29.95	2.02 (2H)	6, 7, 25, 26	
25	125.6	4.89	27, 28	6, 7R, 24, 27	125.6	4.89	27, 28	6, 7R, 24, 27
26	133.6				133.6			
27	26.0	1.65	25, 26, 28		26.0	1.65	25, 26, 28	
28	18.2	1.48	25, 26, 27	24	18.2	1.48	25, 26, 27	24
29	37.7	1.90	1		37.4	1.90	1	
		1.98	9			1.98	9	
30	44.7	2.55	29, 31, 32, 33, 34, 35	32	45.2	2.62	29, 31, 32, 33, 34, 35	
31	148.9				149.5			
32	113.4	4.50	30, 33	12, 16, 30, 33	112.9	4.45	30, 33	30, 33, 37
		4.51	30, 33	12, 16, 30, 33		4.46	30, 33	30, 33, 37
33	17.8	1.60	30, 31, 32		18.26	1.56	31, 32	
34	32.8	1.40			33.5	2.00 (2H)	29, 30, 31, 35, 36	
		1.48						
35	36.8	1.81	34, 36, 37		124.1	5.00	30, 34, 37, 38	37
		1.88						
36	146.9				132.6			
37	110.4	4.62	35, 38	38	25.93	1.65	35, 36	
		4.64	35, 38	38				
38	22.8	1.67			18.31	1.56	35, 36, 37	
39	24.01	1.84 (2H)	23, 41		24.00	1.84 (2H)	23, 41	
40	125.1	5.08	23, 39, 42, 43	42	125.1	5.08	23, 39, 42, 43	42
41	132.8				132.8			
42	25.9	1.64	40, 41, 43		25.9	1.64	40, 41, 43	
43	17.7	1.57	40, 41, 42		17.7	1.57	40, 41, 42	

[a]Recorded in CD_3OD with 0.1% TFA at 125 MHz. Values are reported to two decimal places where necessary to distinguish closely spaced resonances. [b]Recorded in CD_3OD with 0.1% TFA at 500 MHz. [c]Carbons that correlate with the proton resonance. [d]Resonance in the proton column irradiated. [e]196.0, 195.9, 195.6, 195.4, 195.3, 194.1 These may be interchanged.

The organic extract from G. ovalifolia leaves was also active against HIV in vitro. The activity was tracked through a series of chromatographic separations to guttiferone E (5). During the final purification of 5 a related benzophenone derivative, isoxanthochymol (6),[16,17,18] was also isolated. Guttiferone E (5), $C_{38}H_{50}O_6$ (*m/z* 602.3622), had ^1H and ^{13}C NMR spectra virtually identical to those of garcinol (also named camboginol) (8).[22,23,24,25] However, the optical rotation of 5, $[\alpha]_D$ = +101°, was opposite in sign to that reported for 8, $[\alpha]_D$ = -125.[25] Previous NMR studies of 8 resulted in some incompletely or incorrectly assigned resonances; we have assiduously assigned all ^1H and ^{13}C NMR signals for compound 5 (Table 3). Guttiferone E (5) could be converted to isoxanthochymol (6) by acid catalyzed addition of the C1 enol to the terminal methylene in the side



chain. Guttiferone E (**5**) is thus the optical antipode of garcinol (**8**) and a double bond isomer of xanthochymol (**7**). A previous investigation of the stem bark of G. ovalifolia reported the presence of large quantities of xanthochymol (**7**) and trace amounts of isoxanthochymol (**6**).[13] The NMR spectral data of these compounds were not provided; however, the authors did compare the material they isolated with "authentic samples" obtained from another laboratory. It appears that either the benzophenone composition of G. ovalifolia varies between the leaves and the bark or that guttiferone E (**5**) was mistakenly identified as xanthochymol (**7**) in the previous report.[13]

While the absolute stereochemistry of guttiferone E (**5**) was established by its conversion to **6**, the absolute stereochemistries of **1-4** have not been fully defined. On the basis of biogenetic considerations and optical rotation data, they have been drawn with the same absolute stereochemistry as **5**. Guttiferone B (**2**) had a negative optical rotation, but its unique equatorial substituent at C6 may account for this.

The anti-HIV active extract of leaves from Clusia rosea was found to contain guttiferone E (**5**) and xanthochymol (**7**) as the active principles. These double bond isomers occured as an inseparable mixture, but the structures were deduced by comparison of the spectral data of the mixture with the values for **5** and **7**.[17,18,19] ^{13}C NMR resonances of the mixture were virtually identical to those for **5** and **7** and all HMBC correlations were fully consistent with these structures. Other benzophenone derivatives are known from Clusia species[26,27,28] and C. rosea has previously been reported to contain xanthochymol (**7**).[29]

The pure compounds **1**, **2**, **5**, and **6** and the mixture of **3** and **4** were tested in the NCI's primary anti-HIV screen. All of the benzophenone derivatives showed a similar level of activity, except for the cyclized derivative **6**, which was inactive. The active compounds inhibited the cytopathic effects of in vitro HIV infection in human

Table III. NMR Data for Guttiferone E (5) and Isoxanthochymol (6)

	Guttiferone E (5)				Isoxanthochymol (6)			
Position	C[a]	H[b]	HMBC[c]	NOE[d]	C[a]	H[b]	HMBC[c]	NOE[d]
1	194.8				172.4			
2	118.6				110.6			
3	194.0				196.1			
4	69.4				70.4			
5	49.9				47.4[e]			
6	48.0	1.47, m	4, 5, 7, 8, 23, 24, 25	7RS, 23, 24	47.5[e]	1.53, m	4, 5, 7, 8, 23, 24, 25	7RS, 23, 24RS
7	43.3	2.06 proS, dd (14.1, 7.2)	1, 6, 8		38.8	1.81 proS, dd (14.2, 7.2)	1, 5, 8, 24	6, 7R, 23
		2.21 proR, dd (14.0, 1.0)		7S		2.51 proR, dd (14.2, 0.9)	1, 5, 8, 9, 24	7S, 29S
8	59.8				49.6			
9	208.9				209.9			
10	195.8				194.1			
11	129.7				131.1			
12	117.2	7.22, d (2.2)	10, 13, 14, 16		116.1	7.25, d (2.2)	10, 13, 14, 16	
13	146.2				146.4			
14	152.5				152.5			
15	115.0	6.69, d (8.3)	11, 13, 14		115.5	6.71, d (8.3)	11, 13, 14	
16	125.2	7.00, dd (8.3, 2.2)	10, 12, 14		124.3	7.04, dd (8.3, 2.2)	10, 12, 14	
17	27.0	2.48 proS, dd (13.3, 4.6)		17R, 18, 23	26.3[f]	2.45 proS, dd (13.5, 5.7)	3, 4, 18, 19	17R, 23
		2.67 proR, dd (13.3, 9.4)	4, 5, 9, 18, 19	17S, 18, 21, 22,		2.59 proR, dd (13.5, 8.2)	4, 9, 18, 19	17S, 21, 22
18	121.5	4.98, m		17RS, 20	121.2	4.88, m		12, 16, 17RS, 20
19	135.3				135.2			
20	26.4	1.71, d (1.0)	18, 19		26.5[f]	1.58, s	18, 21	
21	18.5[e]	1.65, s	18, 19		18.3[g]	1.66, s		
22	23.2	1.12, s	5, 6, 23	17R, 23, 24	22.7	1.13, s	4, 5, 6, 23	6, 17R, 23, 24R
23	27.3	0.95, s	4, 5, 6, 22	6, 17S, 22, 24	27.1	0.99, s	4, 5, 6, 22	6, 7, 17S, 22
24	30.5	2.06 (2H), m	7, 25, 26		30.5	2.14 proR, m		22
						2.58 proS, m	6, 7, 25, 26	24R, 28
25	125.6	4.88, m		6, 27	126.4	4.93, m		7S, 24RS,27
26	133.5				133.8			
27	25.9[f]	1.65, s	25, 26		26.2[h]	1.70, s	25, 26, 28	
28	18.3[e]	1.52, s	24, 25, 26		18.1	1.58, s	27	
29	36.6	1.71 proS, dd (13.8, 3.7)	9, 31, 34	29SR 30	30.2	1.62 proR, dd (13.8, 2.6)	1, 7, 8, 30, 31, 34	7S, 29S, 30
		2.23 proR, dd (13.8, 10.6)	1, 30	29S		1.96 proS, dd (13.8, 13.5)	7, 8, 9, 30, 31, 34	7R, 29R, 32
30	45.9	2.34, m	29, 31, 32, 33	29S, 32EZ, 34, 35, 38	41.1	1.88, m		33
31	150.0				87.2			
32	112.5	4.35 proZ, d (2.4)	30, 33		22.2	0.83, s	30, 31, 33	29S, 30, 33, 34RS
		4.54 proE, dd (2.4, 1.2)	30, 33					
33	18.2	1.73, s	30, 31, 32		28.4	1.26	30, 31, 32	30, 32, 34S, 38
34	33.9	2.01 (2H), m	29, 30, 31, 35, 36		31.0	1.82 proR, m		29R, 32, 33, 34S
						2.11 proS, m	29, 30, 31, 35, 36	33, 34R 38
35	123.9	4.98, m		30, 32E, 33, 37	122.7	5.11, ddd (6.5, 6.5, 1.2)	30, 34, 36, 37	30, 34RS, 37
36	132.8				135.1			
37	26.0[e]	1.63, d (1.2)	35, 36, 38		26.0[h]	1.73, s	35, 36	
38	18.1	1.57, d (1.0)	35, 36, 37		18.7[g]	1.65, s		

[a]Recorded in CD_3OD with 0.1% TFA at 125 MHz. [b]Recorded in CD_3OD with 0.1% TFAat 500 MHz. [c]Carbons that correlate with the proton resonance. [d]Resonance in the proton column irradiated. [e,f,g,h]May be interchanged within a column.

lymphoblastoid CEM-SS cells, with EC_{50} values of 1-10 μg/ml, while cytotoxicity occured at concentrations greater than 50 μg/ml. However, there were no indications of a corresponding decrease in indices of viral replication (production of reverse transcriptase, p24 and syncytia).[32]

EXPERIMENTAL

General: A description of the equipment and instrumentation used in these studies has been provided in previous reports.[30,31] Final HPLC purifications were effected on Rainin Dynamax^R C_8 and C_{18} columns (1.0 x 25 cm) monitored at 300 nm.

Isolation of guttiferones A-D (1-4) from *Symphonia globulifera*. Samples of the tropical tree S. globulifera were collected in Ndakan Gorilla Study Area of the Central African Republic in March 1988. Voucher specimens from this collection are maintained at the Smithsonian Institution Museum of Natural History, Botany Department. Dried plant material was ground in a Wiley mill and stored at -20° C prior to extraction. The root sample of S. globulifera (378 g) was percolated overnight in 1:1 CH_2Cl_2/MeOH and then washed with 100% MeOH. Removal of the combined solvents under reduced pressure provided 29.7 g of organic extract which was active in the primary anti-HIV screen. A 15 g portion of this extract was partitioned between 300 ml EtOAc and 300 ml of H_2O. The EtOAc layer was removed and extracted with 2 x 100 ml aliquots of H_2O. The combined H_2O layers were washed with 2 x 100 ml aliquots of EtOAc. The combined EtOAc layers were evaporated to give 8.8 g of HIV-inhibitory material. The H_2O soluble fraction was inactive. The EtOAc fraction was dissolved in EtOAc/MeOH, adsorbed onto 25 g of Celite^R and the solvent removed under vacuum. This coated Celite^R was loaded onto the top of a 4 x 21 cm column of diol bonded phase packing and eluted with CH_2Cl_2/EtOAc/MeOH mixtures. Anti-HIV activity was concentrated in the first 3 fractions (eluted with CH_2Cl_2/10% EtOAc); these fractions were combined (2.06 g total) and rechromatographed on a 2.5 x 18 cm diol column eluted with CH_2Cl_2. The active fractions (268 mg total) were combined and passed through a 2.5 x 18 cm C_{18} column. The activity eluted with MeOH/H_2O 9:1 and 100% MeOH and individual column fractions were combined based upon their TLC profiles on phenyl bonded phase plates developed with MeOH/H_2O 7:3. Final purification was achieved by C_8 HPLC using MeOH/4%H_2O/0.01% TFA to give guttiferone A (1) (19 mg), guttiferone B (2) (17 mg) and an inseparable mixture of guttiferone C (3) and guttiferone D (4) (16 mg total).

Isolation of guttiferone A (1) from *Garcinia livingstonei*. Samples of G. livingstonei fruit were collected in the Iringa Region, Mufindi District of Tanzania in December, 1988. Dried, ground plant material (212 g) was extracted as described above to generate 35.8 g of extract. A 1.0 g portion of crude extract was subjected to vacuum liquid chromatography (VLC) on a 6.8 x 3.2 cm C_{18} column eluting with H_2O/MeOH mixtures. The material that eluted with 100% MeOH (472 mg) was permeated through a 2 x 150 cm column of Sephadex LH-20 with hexane/CH_2Cl_2/MeOH (2:5:1). The active fractions were combined (104 mg total) and purified by C_{18} HPLC with 24:1 CH_3CN/H_2O to give 52 mg of guttiferone A (1).

Isolation of guttiferone E (5) and isoxanthochymol (6) from *Garcinia ovalifolia*. Samples of G. ovalifolia were collected in the Ndakan Gorilla Study Area of the Central African Republic in March 1988. The dried, ground leaves (308 g) were extracted as described above to give 24 g of extract. A 5.0 g portion of the extract was subjected to a solvent/solvent partitioning protocol which concentrated the anti-HIV activity in the hexane (920 mg) and CCl_4 (959 mg) soluble fractions. The active fractions were combined and chromatographed on Bio-Beads S-X4 (8 x 75 cm), eluting with 2:4:1 hexane/CH_2Cl_2/EtOAc. Active fractions were combined and passed through a 2.5 x 150 cm column of Sephadex LH-20 with 1:1 CH_2Cl_2/MeOH to give a single active fraction (682 mg). This material was purified by C_{18} HPLC with 24:1 CH_3CN/H_2O to give 179 mg of guttiferone E (5) and 10 mg of isoxanthochymol (6).

Isolation of guttiferone E (5) and xanthochymol (7) from *Clusia rosea*. Samples of C. rosea were collected in the Dominican Republic. The dried, ground leaves (611 g) were extracted as described above to give 31.0 g of crude extract. A 5.5 g portion of the extract was partitioned, concentrating the anti-HIV activity in the CCl_4 soluble fraction. The active fraction (1.0 g) was submitted to sequential gel permeation chromatography on Bio-Beads S-X4 (8 x 75 cm, eluting with 2:4:1 hexane/CH_2Cl_2/EtOAc) and Sephadex LH-20 (1 x 90 cm, eluting with 2:5:1 hexane/CH_2Cl_2/MeOH). The resulting active fraction was subjected to HPLC (C_{18}, 1 x 25 cm, eluting with 97:3 CH_3CN/H_2O) to yield an inseparable mixture (17 mg) of guttiferone E (5) and xanthochymol (7).

Conversion of guttiferone E (5) to isoxanthochymol (6). Guttiferone E (5, 18 mg) was refluxed in 20 ml of toluene with 50 μl conc. HCl for 30 min. The reaction mixture was washed with 2 x 25 ml H_2O and the solvent was then removed *in vacuo*. The residue was suspended in MeOH and purified by C_{18} HPLC with 96:4 acetonitrile/H_2O to give 10 mg of a compound that was identical in all respects, including $[\alpha]_D$, to isoxanthochymol (6).

Guttiferone A (1). $[\alpha]_D$ = +34° ($CHCl_3$, *c* 1.7); EI-HRMS: *m/z* 602.3613, $C_{38}H_{50}O_6$ requires 602.3607; UV λ_{max} (MeOH) 280 (ε 27,300), 228 (ε 22,500) nm; IR (film) υ_{max} 3350, 2968, 1732, 1644, 1519, 1440, 1362, 1290 cm^{-1}.

Guttiferone B (2). $[\alpha]_D$ = -44° ($CHCl_3$, *c* 0.5); EI-HRMS: *m/z* 670.4184, C_{43} $H_{58}O_6$ requires 670.4233; UV λ_{max} (MeOH) 280 (ε 25,300), 228 (ε 21,000) nm; IR (film) υ_{max} 3459, 2960, 1732, 1640, 1537, 1435, 1291, 1193 cm^{-1}.

Guttiferone C (3) and Guttiferone D (4). The mixture was characterized as: $[\alpha]_D$ = +92° ($CHCl_3$), *c* 0.9); EI-HRMS: *m/z* 670.4274, $C_{43}H_{58}O_6$ requires 670.4233; UV λ_{max} (MeOH) 280 (ε 22,300), 228 (ε 18,800) nm; IR (film) υ_{max} 3350, 2970, 1726, 1640, 1440, 1376, 1290, 1117 cm^{-1}.

Guttiferone E (5). $[\alpha]_D$ = +101° ($CHCl_3$, *c* 0.5); EI-HRMS: *m/z* 602.3622, $C_{38}H_{50}O_6$ requires 602.3607; UV λ_{max} (MeOH) 280 (ε 22,600), 228 (ε 19,200) nm; IR (film) υ_{max} 3460, 2960, 1732, 1644, 1442, 1376, 1291, 1193 cm^{-1}.

Isoxanthochymol (6). $[\alpha]_D$ = +181° (EtOH, *c* 0.6); EI-HRMS: *m/z* 602.3594, $C_{38}H_{50}O_6$ requires 602.3607; UV λ_{max} (MeOH) 310 (ε 12,000), 277 (ε 24,000), 233 (ε 21,200) nm; IR (film) υ_{max} 3480, 2960, 1728, 1668, 1595, 1440, 1373, 1290, 1123 cm^{-1}.

Anti-HIV assay. DMSO solutions of compounds 1-6 were tested in the XTT based in vitro anti-HIV assay, experimental details of which have been reported previously.[4]

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