Anti-AIDS Agents. 46.[1] Anti-HIV Activity of Harman, an Anti-HIV Principle from *Symplocos setchuensis*, and Its Derivatives

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Matairesinol (**1**) and harman (**5**), identified from *Symplocos setchuensis*, were found to inhibit HIV replication in H9 lymphocyte cells. Anti-HIV evaluation of 28 derivatives of **5** revealed that compound **19** showed potent activity with EC_{50} and therapeutic index values of 0.037 μM and 210, respectively.

Symplocos setchuensis Brand (Symplocaceae) is indigenous in southern China; however, its chemical constituents have not been investigated. In our continuing anti-HIV screening of plant extracts, the EtOH extract of the stems of *S. setchuensis* demonstrated significant activity (EC_{50} < 20 μg/mL, TI > 5). Subsequent bioassay-directed fractionation of this extract identified matairesinol (**1**) and harman (**5**) as anti-HIV principles.

The ethanolic extract was absorbed on Celite 521 and eluted successively with hexane, $CHCl_3$, EtOAc, and MeOH. Each fraction then was chromatographed repeatedly on Silica gel and ODS columns. Compound **1** and (+)-pinoresinol (**2**) were the major components in the $CHCl_3$ fraction and were obtained as a mixture in a 5:2 ratio. Compound **2** was separated by acetylation of the mixture, isolation of the diacetate, and deacetylation. Compound **1** was isolated and identified as the aglycone of matairesinoside (**3**), which was isolated together with **1** and **2**. The diacetate (**1a**) of **1** was obtained by acetylation. (+)-Isolariciresinol (**4**), betulinic acid, oleanolic acid, stigmasterol glucoside, and stigmasterol also were identified from the $CHCl_3$ fraction. After the isolation of the above compounds, the remainder of the $CHCl_3$ fraction was washed with 2 N Na_2CO_3 and 2 N NaOH successively and then extracted with 2 N HCl to give an alkaloidal fraction. Harman (**5**) was identified in this fraction by HPLC analysis. Lupeol and stigmasterol were isolated and identified from the hexane extract, and (+)-pinoresinol-β-D-glucoside (**6**) was isolated and identified from the MeOH extract.

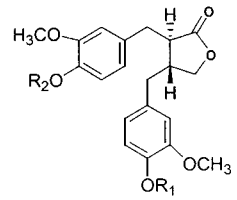
All compounds and AZT were examined for HIV replication inhibition activity in H9 lymphocytes (Table 1). Compounds **1**, **1a**, and **5** demonstrated potent anti-HIV activity, with EC_{50} and therapeutic indexes (TI) values of 2.0, 6.9, and 10.7 μM and 11.0, 7.1, and 10.4, respectively. The other lignanolides (**2**, **3**, **4**, and **6**) were inactive. We previously reported the HIV replication inhibitory activity of betulinic acid and oleanolic acid;[2] however, lupeol, stigmasterol, and stigmasterol glucoside isolated at the same time were inactive.

Some lignans structurally similar to compound **1**, such as (−)-arctigenin and (−)-trachelogenin, are known to inhibit replication of HIV-1 in infected human cell systems.[3] Eich et al. examined **1** and related compounds against HIV-1 integrase and reported that **1** was not active against this enzyme.[4] Now, our results indicate that compounds **1** and **1a** might regulate other steps in the HIV replication pathway. Hence, compounds **1** and **1a** are possible lead compounds for the design of novel HIV-replication inhibitors.

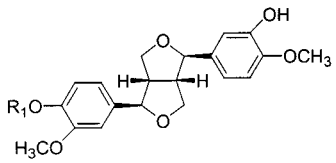
Our group is first to report anti-HIV activity with β-carbolines, as shown with **5** and with 1-methoxycanthin-6-one, which was isolated previously in our laboratory from *Leitneria floridana* Chapman.[1] The results reported herein promoted our preparation and evaluation of 28 derivatives of **5** (Table 2).

As shown in Table 2, **19** (*N*-butylharmine) was the most potent compound (EC_{50} = 0.037 μM) in this series and possessed a good therapeutic index of 210. Compounds **9**

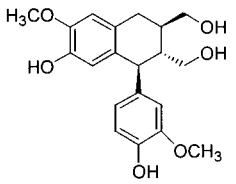
Table 1. Inhibition of HIV-1 Replication in H9 Lymphocytic Cells



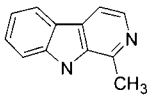
1 : R_1 = H, R_2 = H
1a : R_1 = Ac, R_2 = Ac
3 : R_1 = H, R_2 = glc



2 : R_1 = H
6 : R_1 = glc



4



5

compound	IC_{50} (μM)[a]	EC_{50} (μM)[b]	TI[c]
1	21.9	2.0	11.0
1a	49.3	6.9	7.1
2	67.0	NS	
3	>192	NS	
4	>277	NS	
5	111.5	10.7	10.4
6	>279	NS	
AZT	1875	0.045	41667

[a] Concentration that inhibits uninfected H9 cell growth by 50%. [b] Concentration that inhibits viral replication by 50%. [c] Therapeutic index = IC_{50}/EC_{50}; NS, no suppression,

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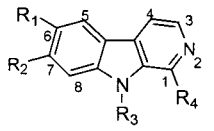
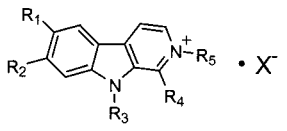
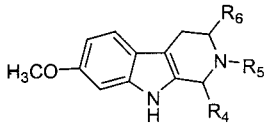
Table 2. Inhibition of HIV-1 Replication in H9 Lymphocytic Cells by β-Carbolines



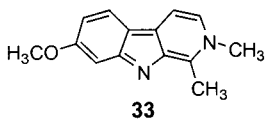
A



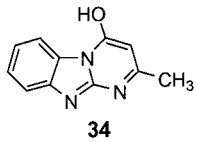
B



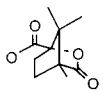
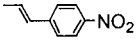
C



33



34

Com	skele	R_1	R_2	R_3	R_4	R_5	R_6	X	IC_{50} (μM)[a]	EC_{50} (μM)[b]	TI[c]
5	A	H	H	H	CH_3				111.5	10.7	10.4
7	A	H	H	H	H				141.7	44.1	3.2
8	A	H	OH	H	CH_3				108.1	11.1	9.7
9	A	H	OCH_3	H	CH_3				26.4	0.52	50.8
10	A	H	OC_2H_5	H	CH_3				25.9	6.5	4.0
11	A	H	OAc	H	CH_3				106.7	17.3	6.2
12	A	H	$OCH(CH_3)_2$	H	CH_3				86.7	NS	-
13	A	H	$O(CH_2)_5CH_3$	H	CH_3				8.2	NS	-
14	A	H	$O(CH_2)_9CH_3$	H	CH_3				5.9	NS	-
15	A	H	$O(CH_2)_{15}CH_3$	H	CH_3				42.7	NS	-
16	A	H		H	CH_3				58.9	NS	-
17	A	H	OCH_3	CH_3	CH_3				10.1	1.1	9.2
18	A	H	OCH_3	C_2H_5	CH_3				8.5	0.35	24.2
19	A	H	OCH_3	$(CH_2)_3CH_3$	CH_3				7.8	0.037	210
20	A	H	OCH_3	H					15.4	NS	-
21	A	H	H	H	CH_3 3,4-dihydro				110.3	32.0	3.4
22	A	Br	OCH_3	H	CH_3				74.0	13.2	5.6
23	B	H	OCH_3	H	CH_3	CH_3		I	49.6	8.6	5.8
24	B	Br	OCH_3	H	CH_3	H		Br	5.9	0.22	26.8
25	B	H	OCH_3	C_3H_7	CH_3	CH_3		Br	53.2	23.8	2.2
26	B	H	OCH_3	H	CH_3	C_3H_7		I	55.4	9.6	5.8
27	B	H	OH	H	CH_3 3,4-dihydro	H		Br	97.1	NS	-
28	B	H	OH	H	CH_3 3,4-dihydro	H		Cl	87.1	NS	-
29	B	H	H	H	CH_3	H		CH_3SO_4	77.5	10.3	7.5
30	C	H	OCH_3	H	CH_3	NO	H_2		56.5	7.1	8.0
31	C	H	H	H	$(CH_2)_7CH_3$	H	COOH		60.4	NS	-
32	C	H	H	H	$CH(C_2H_5)_2$	H	COOH		ND	ND	-
33									70.9	4.5	15.8
34									118.1	NS	-
AZT									1875	0.045	41667

[a] Concentration that inhibits uninfected H9 cell growth by 50%. [b] Concentration that inhibits viral replication by 50%. [c] Therapeutic index = IC_{50}/EC_{50}; NS, no suppression; ND, not dissolved.

(harmine), **18** (*N*-ethylharmine), **24** (6-bromoharmine hydrobromide), and **33** also were potent (EC_{50} = 0.52, 0.35, 0.22, and 4.5 μM) and possessed a moderate therapeutic index (50.8, 24.2, 26.8, and 15.8), respectively. The remaining compounds were less potent than **5**.

By comparing the anti-HIV inhibitory activities of the compounds in Table 2, the following trends became evident. (a) Introduction of a methoxy group at the 7-position of harmine (**9**) led to enhanced anti-HIV activity compared with unsubstituted harman (**5**). The 7-hydroxy (**8**), the 7-ethoxy (**10**), and the 7-acetoxy (**11**) derivatives possessed activities in the same magnitude as the parent compound (**5**); however, the isopropoxy (**12**), the hexyloxy (**13**), the decyloxy (**14**), the hexadecyloxy (**15**), and the 7-camphanoyl (**16**) derivatives were inactive. Thus, the substituent at the 7-position can affect the activity of this series of compounds dramatically. (b) Compared with **5**, compound **7**, which lacks a methyl group at the 1-position, showed ca. 4-fold

lower activity. Thus, the 1-methyl group of **5** seems to be important for activity in this series of compounds. (c) 3,4-Dihydro β-carboline (**21**), produced by hydrogenation of **5,** had lower anti-HIV replication activity compared with the unsaturated compound. (d) Bromination of **9** at C-6 gave 6-bromoharmine hydrobromide (**24**); this water-soluble derivative showed the highest potency in this series of compounds (EC_{50} 0.22 μM, TI 26.6). The corresponding free base (**22**) was not as potent as **24**, perhaps due to limited solubility in the assay buffer and media. (e) The fully conjugated derivative (**33**) retained some activity. (f) Compound **34**, which possesses a benzimidazole in place of the indole normally found in β-carbolines, displayed no activity. Thus the β-carboline structure may be important for the anti-HIV activity of these compounds. (g) Alkylation of the indole nitrogen (**17**, **18**, and **19**) increased the anti-HIV activity. Compound **19** (*N*-butyl) was more active than **18** (*N*-ethyl), indicating that the length of the alkyl chain at this position is important to activity.

In summary, within the series of β-carboline derivatives (**5**, **7**−**34**), substitution of the parent compound harman (**5**) with 1-methyl, 7-methoxy, or an alkyl group at the indole nitrogen led to increased anti-HIV activity. Synthesis of additional analogues of this type is currently ongoing in our laboratory in an effort to identify more potent anti-HIV inhibitors.

Experimental Section

Plant Materials. *S. setchuensis* was collected in the Shichuan Province, People's Republic of China.

Extraction and Isolation. The 95% EtOH extract (76.5 g) of the stems of *S. setchuensis* was adsorbed on Celite 521 and eluted successively with hexane, $CHCl_3$, EtOAc, and MeOH. Each fraction then was chromatographed repeatedly on silica gel and ODS columns. The $CHCl_3$ fraction (19.4 g) was chromatographed on a silica gel column using $CHCl_3$−MeOH (1:0 → 0:1) as eluents to afford fractions 1−9. Fraction 2 gave betulinic acid (1 mg), oleanolic acid, and a mixture of matairesinol (**1**) and (+)-pinoresinol (**2**) in a 5:2 ratio. Compound **2** (10 mg) was isolated by acetylation of the mixture, isolation of the diacetate, and deacetylation. Compound **1** was isolated and identified as the aglycone of matairesinoside (**3**). The diacetate (**1a**) was generated by acetic anhydride−pyridine acetylation of **1** at room temperature. Fractions 3, 4, and 5 afforded isolariciresinol (**4**) (18.1 mg), stigmasterol glucoside, and matairesinoside (**3**) (550 mg), respectively. The MeOH fraction afforded (+)-pinoresinol-β-glucoside (**6**) (3 mg), and the hexane fraction afforded lupeol and stigmasterol. The structures of **1**−**4**, **6**, and betulinic acid were confirmed by comparing their physical and spectral data with those reported in the literature.[5−9] Oleanolic acid, stigmasterol glucoside, and stigmasterol were identified by direct TLC comparisons with authentic samples. After removal of the above compounds, the remainder of the $CHCl_3$ fraction was washed with 2 N Na_2CO_3 and 2 N NaOH successively and then extracted with 2 N HCl to give an alkaloid-containing fraction. After purification with preparative TLC, harman (**5**) was confirmed by HPLC analysis, where a peak corresponding to harman was detected in this fraction [Alltima C_{18} (particle size 5 μm), 4.6 × 250 mm; ammonium acetate 25 mM adjusted with H_3PO_4 (pH 4.0): MeOH (40:60, v/v) at a flow rate of 1.0 mL/min; UV detection at 287 nm; t_R (min) 12.3 (harman)]. Compound **5** was also identified by direct TLC comparison with an authentic sample [R_f 0.5, $CHCl_3$−MeOH (10:1)]. Harman was reported previously from the related species *S. racomosa* Roxb.[10]

Compounds **7**, **21**, and **27** were obtained from Aldrich, Inc. (Milwaukee, WI). The preparation of the remaining compounds (**5**, **8**−**20**, and **22**−**34**) has been described in our previous paper.[11]

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References and Notes

(1) For part 45, see: Xu, Z.; Chang, F. R.; Wang, H. K.; Kashiwada, Y.; McPhail, A. T.; Bastow, K. F.; Tachibana, Y.; Cosentino, M.; Lee, K. H. Anti-HIV Agents 45. Two New Sesquiterpenes, Leitneridanins A and B, and the Cytotoxic and Anti-HIV Principles from *Leitneria floridana*. *J. Nat. Prod.* **2000**, *63*, 1712.
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