# Natural anti-HIV agents. Part IV. Anti-HIV constituents from Vatica cinerea

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# Natural Anti-HIV Agents. Part IV. Anti-HIV Constituents from *Vatica cinerea*[1]

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In a continuing search for anti-HIV compounds from plants of Vietnam, 19 compounds, including a new triterpene, were isolated from an extract of the leaves and stem of *Vatica cinerea*. The new triterpene was determined to be a cycloartane triterpenoid with 29 skeletal carbons and was assigned the name vaticinone (**1**). The known triterpenes included three cycloartanes, a lanostane, two dammaranes, three lupanes, an ursane, and an oleanane. A chlorophyll isolate was identified as pheophorbide *a* (**13**). The majority of the triterpenes, the sesquiterpene, 1-hydroxycyclocolorenone, and pheophorbide *a* showed anti-HIV activity, with the chlorophyll being the most active, demonstrating an $IC_{50}$ value of 1.5 $\mu$g/mL (2.5 $\mu$M), while being completely devoid of toxicity up to a concentration of 20 $\mu$g/mL (33.8 $\mu$M). Vaticinone (**1**) was found to inhibit the replication of HIV-1, with an $IC_{50}$ value of 6.5 $\mu$g/mL (15.3 $\mu$M; selective index = 1.4). The structures of these isolates were determined by spectral data including 1D and 2D NMR spectra.

A recent report indicates that approximately 20 million people have died from AIDS (acquired immunodeficiency syndrome) and that an estimated 36 million individuals are infected with the human immunodeficiency virus (HIV).[2] AIDS is presently the leading cause of death in Africa and the fourth leading cause of death worldwide. As part of an International Cooperative Biodiversity Group (ICBG) involving the collaboration of institutions in Vietnam, Laos, and the United States,[3] we have established a natural products antiviral drug discovery program targeting specifically HIV.

During an initial screen, a chloroform-soluble extract prepared from the leaves and twigs of *Vatica cinerea* King (Dipterocarpaceae) collected in the Cuc Phuong National Park, Vietnam, was shown to inhibit HIV-1 replication by 86% at 20 $\mu$g/mL, while being devoid of cytoxicity at this concentration. A search of the literature did not uncover any prior phytochemical or pharmacological reports with this plant. Therefore, this material was considered a promising candidate for bioassay-directed fractionation studies aimed to identify novel anti-HIV constituents.

Accordingly, bioassay-guided phytochemical separation led to the isolation of 19 compounds, including 12 triterpenes, of which one has a new structure. The current paper describes the isolation, identification, and biological evaluation of the anti-HIV compounds from this species.

## Results and Discussion

Bioassay-directed fractionation of the $CHCl_3$-soluble fraction of the dried leaves and twigs of *V. cinerea* resulted in the isolation of 19 compounds that included 12 triterpenes. Of the triterpenes, one was found to possess a new structure. The new triterpene, designated as vaticinone (**1**), was determined to possess a cycloartane skeleton containing 29 carbons. The known triterpenes were identified by comparison with literature spectral data as three cycloartanes, (23*E*)-27-*nor*-3$\beta$-hydroxycycloart-23-en-25-one (**2**),[4] mangiferonic acid (**3**),[5] and dihydroschizandronic acid (**4**);[6] a lanostane, (24*E*)-3-oxo-lanosta-8,24-dien-26-oic acid (**5**);[7] two dammaranes, dammara-20,25-dien-3$\beta$,24-diol (**6**)[8,9] and (23*E*)-dammara-20,23-dien-3$\beta$,25-diol (**7**);[9] three lupanes, betulinic acid (**8**),[11] betulin (**9**),[10] and betulonic acid (**10**);[11] an ursane, ursolic acid (**11**);[12] and an oleanane, erythrodiol (**12**) (Chart 1).[13] In addition to the above triterpenes, seven other known compounds were also isolated. These were identified as pheophorbide *a* (**13**),[14] 3$\beta$-glucositosterol,[15] (3*R*,6*R*,7*E*)-3-hydroxy-4,7-megastigmadien-9-one,[16] *N*-butylbenzenamine,[17] hydroxydihydrobovolide,[18] eudesm-4(15)-ene-1$\beta$,6$\alpha$-diol,[19] and 1-hydroxycyclocolorenone (**14**).[20]

Vaticinone (**1**) was obtained as a white powder with a molecular formula of $C_{29}H_{44}O_2$ based on the HREIMS data ($[M]^+$ *m/z* 424.3350, calcd 424.3341). The $^{13}C$ NMR and DEPT spectra determined the 29 skeleton carbons in **1** as composed of six methyl, 10 methylene, four methine, two olefinic, five quaternary, and two quaternary carbonyl carbons (Table 2). Except for the carbonyls, none of other carbons in **1** were found to be oxygenated. Characteristic $^1H$ NMR signals for methyl groups at $\delta$ 0.88 (3H, s), 0.89 (3H, d, $J$ = 6.4 Hz), 1.00 (3H, s), 1.03 (3H, s), 1.08 (3H, s), and 2.24 (3H, s) were observed. A pair of characteristic doublet signals in the high-field of the $^1H$ NMR spectrum for a methylene group [$\delta$ 0.56 (3H, ABd, $J$ = 4.3 Hz) and 0.77 (3H, brd, $J$ = 4.1 Hz)] were discerned, in addition to the multi-methylene signals in the upfield regions of both the $^1H$ and the $^{13}C$ NMR spectra, which indicated **1** to be a cycloartane triterpene derivative (Tables 1 and 2). The only two oxygenated functional groups found in **1** were assigned as quaternary carbonyl carbons. One of the carbonyl carbons formed an $\alpha,\beta$-unsaturated ketone by conjugating with the one and only double bond of **1** [$\delta_H$ 6.78 (1H, ddd, $J$ = 15.1, 8.7, 6.2 Hz), 6.06 (1H, brd, $J$ = 15.8 Hz), and $\delta_C$ 198.2 (s), 147.6 (d), 132.6 (d)]. This $\alpha,\beta$-unsaturated ketone was localized in the side chain of **1** by analysis of the $^1H-^1H$ COSY, HMQC, and HMBC NMR spectral data (Figure 1). Further inspection of the HMBC

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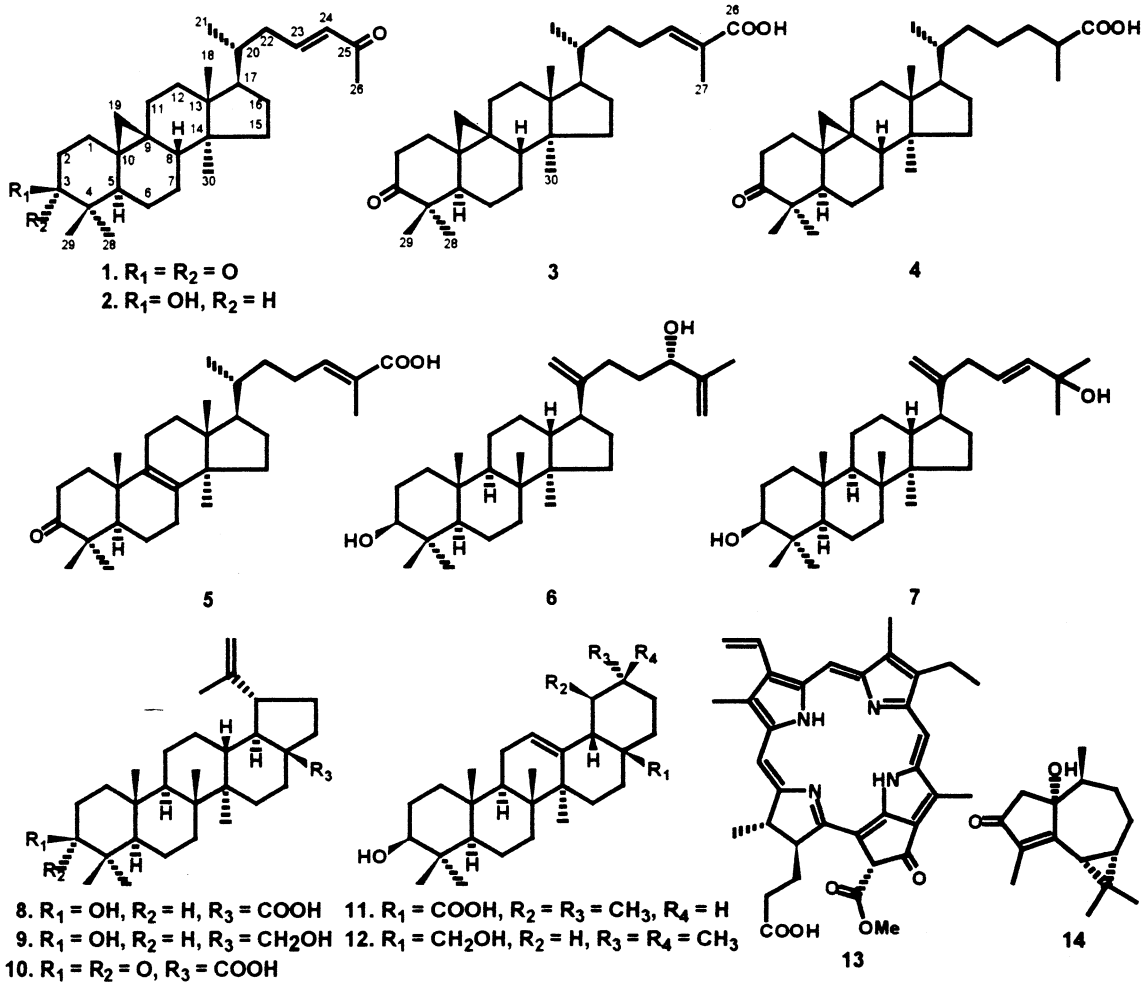
**Chart 1**




spectral data of **1** assigned the remaining carbonyl carbon at C-3. In addition, the double bond of the α,*β*-unsaturated ketone was determined to have an *E*-configuration due to the large coupling constant ($J$ = 15.1) between its two protons. Accordingly, **1** was elucidated as (23*E*)-27-*nor*-cycloart-23-en-3,25-dione and given the trivial name of vaticinone.

Compounds **2**−**5** have previously been reported. However, the structural similarity of these compounds and the fact that **4** and **5** are being reported for the first time as naturally occurring compounds merit some discussion. (23*E*)-27-*Nor*-3*β*-hydroxycycloart-23-en-25-one (**2**), mangiferonic acid (**3**), and dihydroschizandronic acid (**4**) were determined to be triterpenes with structures similar to that of **1**. Analysis of $^1$H and $^{13}$C NMR data of compounds **1**−**5** revealed **2** as a cycloartane triterpene with only 29 carbons on the skeleton. Compounds **3** and **4** were found to be normal cycloartane triterpenoids with 30 skeletal carbons, while **5** was found to be a lanostane triterpenoid. Compound **2** differs from **1** only by a functional group at C-3, which was found to be hydroxylated in **2** instead of carboxylated as in **1**. The reduction of the carboxynyl carbon in **1** to the hydroxyl group in **2** resulted in dramatic upfield shifts at C-2, -4, and -29, in addition to a significant downfield shift at C-28 (Table 2). Compound **3** differs from **1** by its side chain group; the α,*β*-unsaturated ketone in **1** was replaced by an α,*β*-unsaturated carboxylic acid [$\delta_H$ 6.89 (1H, tq, $J$ = 7.7, 1.4 Hz), 6.06 (1H, brd, $J$ = 15.8), and $\delta_C$ 173.5 (s), 145.8 (d), 126.6 (d)] in **3** (Tables 1 and 2). The conjugated double bond in **3** was established to be in the *E*-configuration due to the downfield shift of the double bond proton ($\delta_H$ 6.89) caused by the close proximity of the carbonyl carbon.[21] Compound **4** has been reported as a semisynthetic hydrogenated product of schizandronic acid.[6] The NMR data we recorded with **4** are very similar to those of **3** (Tables 1 and 2), which denoted **4** to be a close structural relative of compound **3**. With no carbon−carbon double bond signals being observed in the NMR spectra, **4** was, therefore, assigned as dihydroschizandronic acid, a hydrogenated mangiferonic acid. Compound **5** is a patented compound, which was synthesized from 24,25-expoxyl-anost-8-en-3*β*-ol (**8**). It has the same molecular formula of $C_{30}H_{46}O_3$ as that of **3**, as confirmed by HRTOFMS (**3**: [M − H]$^+$ *m*/*z* 453.3373, calcd 453.3369; **5**: [M − H]$^+$ *m*/*z* 453.3371, calcd 453.3369). The $^1$H and $^{13}$C NMR spectra revealed that the two compounds (**3** and **5**) have very

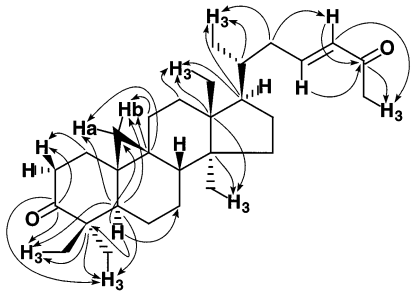


**Figure 1.** Selected HMBC correlations for compound **1** ($CDCl_3$).

**Table 1.** $^1$H NMR Data of Compounds **1**−**5** (500 MHz, $CDCl_3$, *J* in Hz, $\delta$ in ppm)

| proton | 1 | 2 | 3 | 4 | 5 |
|---|---|---|---|---|---|
| H-1α | 1.83 brtd (13.5, 3.6) | | 1.82 brtd (13.4, 4.0) | 1.83 brtd (13.4, 4.4) | |
| H-1*β* | 1.52 m | | | | |
| H-1a | | | | | |
| H-1b | | | | | |
| H-2α | 2.29 ddd (14.0, 4.3, 2.6) | | 2.28 ddd (14.0, 4.3, 2.6) | 2.28 ddd (14.0, 4.3, 2.6) | 2.38 ddd (15.8, 6.8, 3.5) |
| H-2*β* | 2.69 td (13.9, 6.4) | | 2.69 td (13.9, 6.4) | 2.68 td (13.9, 6.4) | 2.55 ddd (15.9, 11.2, 7.2) |
| H-2a | | | | | |
| H-2b | | | | | |
| H-3α | | 3.26 dd (11.0, 4.2) | | | |
| H-5α | 1.69 dd (12.5, 4.2) | | 1.69 dd (12.3, 4.4) | 1.69 dd (12.3, 4.5) | |
| $H_2$-6 | 1.55 overlap | | | | |
| H-7a | 1.40 m | | | | |
| H-7b | 1.12 m | | | | |
| H-8*β* | 1.58 overlap | | | | |
| H-11α | 2.05 m | | | | |
| H-11*β* | 1.15 m | | | 2.02 ddd (14.7, 9.8, 7.2) | |
| $H_2$-12 | 1.62 overlap | | | | |
| $H_2$-15 | 1.35 m | | | | |
| $H_2$-16 | 1.92 m | | | | |
| H-17α | 1.62 overlap | | | | |
| Me-18 | 1.00 s | 0.97 s | 0.97 s | 0.96 s | 0.69 s |
| H-19α | 0.56 ABd (4.3) | 0.32 ABd (4.2) | 0.55 ABd (4.2) | 0.55 ABd (4.3) | |
| H-19*β* | 0.77 brd (4.1) | 0.54 brd (4.2) | 0.76 brd (4.0) | 0.76 brd (4.2) | |
| Me-19 | | | | | 1.06 s |
| H-20*β* | 1.60 overlap | | | | |
| Me-21 | 0.89 d (6.4) | 0.88 d (6.5) | 0.89 d (6.5) | 0.84 d (6.5) | 0.92 d (6.3) |
| H-22a | 2.36 m | 2.35 brdd (14.0, 6.1) | | | |
| H-22b | 1.96 m | | | | |
| H-23 | 6.78 ddd (15.1, 8.7, 6.2) | 6.78 ddd (15.6, 8.7, 6.2) | | | |
| H-24 | 6.06 brd (15.8) | 6.05 brd (15.8) | 6.89 tq (7.7, 1.4) | | 6.88 brt (7.5) |
| H-25 | | | | 2.45 se[a] (6.9) | |
| Me-26 | 2.24 s | 2.23 s | | | |
| Me-27 | | | 1.82 d (0.9) | 1.16 d (6.9) | 1.81 s |
| Me-28 | 1.03 s | 0.95 s | 1.02 s | 1.02 s | 1.04 s |
| Me-29 | 1.08 s | 0.79 s | 1.07 s | 1.07 s | 1.09 s |
| Me-30 | 0.88 s | 0.87 s | 0.88 s | | 0.86 s |

[a] se represents sextet.

**Table 2.** $^{13}$C NMR Data of Compounds **1**−**5** (500 MHz, $CDCl_3$, $\delta$ in ppm)

| carbon | 1 | 2 | 3 | 4 | 5 |
|---|---|---|---|---|---|
| C-1 | 33.4 t | 32.0 t | 33.4 t | 33.4 t | 30.9 t |
| C-2 | 37.5 t | 30.4 t | 37.4 t | 37.5 t | 36.3 t |
| C-3 | 216.6 s | 78.8 d | 216.5 s | 216.8 s | 217.9 s |
| C-4 | 50.2 s | 40.5 s | 50.2 s | 50.2 s | 49.9 s |
| C-5 | 48.4 s | 47.4 d | 48.4 d | 48.4 d | 50.3 d |
| C-6 | 21.5 s | 21.1 t | 21.5 t | 21.5 t | 21.0 t |
| C-7 | 25.8 t | 26.0 t | 25.8 t | 25.8 t | 26.3 t |
| C-8 | 47.9 d | 48.0 d | 47.8 d | 47.9 d | 133.1 s |
| C-9 | 21.0 s | 19.8 s | 21.0 s | 21.0 s | 135.2 s |
| C-10 | 26.0 s | 26.1 s | 26.0 s | 25.9 s | 36.9 s |
| C-11 | 26.6 t | 26.4 t | 26.6 t | 26.7 t | 19.4 t |
| C-12 | 32.6 t | 32.8 t | 32.7 t | 32.8 t | 30.8 t |
| C-13 | 45.4 s | 45.4 s | 45.3 s | 45.3 s | 44.5 s |
| C-14 | 48.8 s | 48.9 s | 48.7 s | 48.7 s | 47.4 s |
| C-15 | 35.5 t | 35.5 t | 35.5 t | 35.5 t | 34.6 t |
| C-16 | 28.2 t | 28.2 t | 28.1 t | 28.1 t | 28.1 t |
| C-17 | 52.2 d | 52.2 d | 52.2 d | 52.3 d | 51.2 d |
| C-18 | 18.1 q | 18.1 q | 18.1 q | 18.1 q | 15.8 q |
| C-19 | 29.6 t | 29.9 t | 29.5 t | 29.6 t | 26.2 q |
| C-20 | 36.1 d | 36.2 d | 35.9 d | 35.9 d | 36.3 d |
| C-21 | 18.6 q | 18.6 q | 18.1 q | 18.2 q | 18.5 q |
| C-22 | 39.6 t | 39.6 t | 25.9 t | 24.0 t | 25.9 t |
| C-23 | 147.6 d | 147.6 d | 34.1 t | 34.0 t | 34.7 t |
| C-24 | 132.6 d | 132.6 d | 145.8 d | 36.0 t | 145.7 d |
| C-25 | 198.2 s | 198.4 s | 126.6 s | 39.4 d | 126.7 s |
| C-26 | 27.0 q | 26.9 q | 173.5 s | 182.9 s | 173.4 s |
| C-27 | | | 11.9 q | 17.0 q | 11.9 q |
| C-28 | 22.2 q | 25.4 q | 22.1 q | 22.2 q | 24.3 q |
| C-29 | 20.8 q | 14.0 q | 20.7 q | 20.7 q | 21.3 q |
| C-30 | 19.3 q | 19.3 q | 19.3 q | 19.3 q | 18.6 q |

similar structures. Compound **5** contained an α,*β*-unsaturated carboxylic acid group [$\delta_H$ 6.89 (1H, brt, $J$ = 7.5), $\delta_C$ 173.4 (s), 145.7 (d), 126.7 (s)], and the conjugated double bond remained in the *E*-configuration. Compound **5** differed from **3** in that the latter contains no cyclopropyl group but has an additional double bond at $\delta$ 135.24 (s) and 133.14 (s). The double bond was assigned as $\Delta^{8,9}$, which resulted in dramatic changes in $^{13}$C NMR chemical shifts at C-1, -10, -11, and -18 in **5**. The structural identification of compounds **2**, **3**, and **5** was confirmed by comparing their spectral data to those reported in the literature.[4,5,7] Since neither the spectral data of **4** nor the $[\alpha]_D$ and $^{13}$C NMR data of **5** have ever been published in the literature, this information is presented in the Materials and Methods section and Tables 1 and 2 of the current report. For the purpose of comparison, the $^{13}$C NMR data of compounds **2** and **3** are also included in Table 2. All other compounds were identified by comparison of their spectral data including NMR with those described in the literature.

A total of 11 anti-HIV compounds were isolated from *V. cinerea*, of which nine (**1**, **2**, **5**−**11**) were triterpenes. A sesquiterpene (**14**) and a chlorophyll derivative (**13**) were also found to be active. The anti-HIV activity and cytotoxicity of these compounds are presented in Table 3. The starting $CHCl_3$ extract of the leaves and stems of the plant inhibited HIV-1 replication by 86% at 20 $\mu$g/mL without any evidence of cytotoxicity. The HIV-1 inhibitory activity of the resulting fractions intensified as fractionation progressed. Peaks of activity were found in F-35 (42% inhibition of HIV-1 replication at 10 $\mu$g/mL without toxicity), F-36 (97% inhibition of HIV-1 replication and 39% cellular viability at 10 $\mu$g/mL), and F-49 (49% inhibition at 5 $\mu$g/mL without toxicity up to 10 $\mu$g/mL). The subsequent preparative HPLC separation of F-36 resulted in 17 frac-

**Table 3.** Anti-HIV Activity and Cytotoxicity of Compounds **1**−**14** Isolated from *Vatica cinerea* King

| compd | name | $CC_{50}$[a] [$\mu$g/mL ($\mu$M)] (HOG.R5) | $IC_{50}$[b] [$\mu$g/mL ($\mu$M)] (HOG.R5) | selectivity index (SI)[c] | status[d] |
|---|---|---|---|---|---|
| **1** | vaticinone | 9.1 (21.5) | 6.5 (15.3) | 1.4 | A |
| **2** | (23*E*)-27-*nor*-3$\beta$-hydroxy-cycloart-23-en-25-one | 5.4 (12.7) | 21% inhibition @ 2.5 $\mu$g/mL (5.9) | | A |
| **3** | mangiferonic acid | nontoxic @ 20 $\mu$g/mL (44.1 $\mu$M) | 19% inhibition @ 20 $\mu$g/mL (44.1 $\mu$M) | | I |
| **4** | dihydroschizandronic acid | nontoxic @ 20 $\mu$g/mL (43.9 $\mu$M) | 0% inhibition @ 20 $\mu$g/mL (43.9 $\mu$M) | | I |
| **5** | (24*E*)-3-oxo-lanosta-8,24-dien-26-oic acid | 6.7 (14.8) | 3.9 (8.6) | 1.7 | A |
| **6** | dammara-20,25-dien-3$\beta$,24-diol | 6.3 (14.3) | 2.7 (6.1) | 2.3 | A |
| **7** | (23*E*)-dammara-20,23-dien-3$\beta$,25-diol | 13.4 (30.3) | 10.0 (22.6) | 1.3 | A |
| **8** | betulinic acid | 16.4 (36.0) | 14.8 (32.5) | 1.1 | A |
| **9** | betulin | 8.6 (19.5) | 6.1 (13.8) | 1.4 | A |
| **10** | betulonic acid | 47.7 (105.1) | 9.7 (21.4) | | A |
| **11** | ursolic acid | 7.2 (15.8) | 6.7 (14.7) | 4.9 | A |
| **12** | erythrodiol | nontoxic @ 20 $\mu$g/mL (45.2 $\mu$M) | 33% inhibition @ 20 $\mu$g/mL (45.2 $\mu$M) | >1 | I |
| **13** | pheophorbide *a* | ~20 $\mu$g/mL (33.8 $\mu$M) | 1.5 (2.5 $\mu$M) | >13 | A |
| **14** | 1-hydroxycyclocolorenone | nontoxic @ 20 $\mu$g/mL (85.5 $\mu$M) | 20.6 (88.0 $\mu$M) | >1 | A |

[a] $CC_{50}$ = concentration mediating a 50% cytotoxic response. [b] $IC_{50}$ = concentration mediating a 50% inhibition of HIV-$1_{IIIB}$ replication. [c] Selectivity index (SI) = CC50/IC50. [d] A = active; I = inactive

tions, most of which exhibited >40% inhibition of HIV-1 infectivity at a concentration of 10 $\mu$g/mL. The broad spread of activity across these consecutive fractions suggested they contain a series of structurally similar compounds. The $^1$H NMR of these fractions revealed that they contain mainly triterpenoids. Indeed, all active compounds (**1**, **2**, **5**−**11**) purified from F-36 were identified as triterpenes (Table 1).

All anti-HIV compounds uncovered except for **5**, **6**, and **13** appeared to demonstrate weak to moderate activity. However, in all cases the low selective index (SI) values obtained would warrant further evaluation of these compounds in the more pathologically relevant H9 T lymphocyte cell line that supports efficient in vitro HIV-1 replication. H9 cells have also been known to be less susceptible to toxicity. This study will be initiated upon the isolation of larger amounts of the compounds of interest. It is noteworthy that six (cycloartane possessing 29 skeletal carbons, lanostane, dammarane, lupane, ursane, and oleanane) of the seven skeletal classes of triterpenes isolated from *V. cinerea* demonstrated some degree of anti-HIV activity. Only the cycloartane compounds with 30 carbon atoms in their skeleton (compounds **3** and **4**) were devoid of activity against the virus (Table 3).

Unlike fraction F-36, fraction F-35 was devoid of triterpenes. The active isolate was determined to be a sesquiterpene (**14**), which showed an anti-HIV $IC_{50}$ value of 20.6 $\mu$g/mL (88.0 $\mu$M) without any evidence of cytotoxicity at 20 $\mu$g/mL (85.5 $\mu$M).

Workup of fraction F-49 resulted in the isolation of an anti-HIV chlorophyll, pheophorbide *a* (**13**), in the form of a nonmetal ion coordinated molecule. This chlorophyll exhibited the most potent anti-HIV activity [$IC_{50}$ = 1.5 $\mu$g/mL (2.5 $\mu$M)] of all the isolates obtained from this plant. It was also completely nontoxic up to a concentration of 20 $\mu$g/mL (33.8 $\mu$M). The anti-HIV activity of pheophorbide *a* (**13**) was more pronounced than that of betulinic (**8**)[22] or ursolic acids (**11**),[23] two established representatives of triterpenoid compounds that possess potent anti-HIV activity.

Pheophorbide *a* is relatively abundant in green plants since it is known to be one of the significant degradation products of chlorophyll *a*. It is formed by the removal of magnesium ion and a phytyl group through the action of chlorophyllase in plants and bacteria under acidic conditions. Pheophorbide *a* derivatives are structurally related to porphyrins and are of current interest as photosensitizers for use in photodynamic therapy (PDT), a modality developed to treat cancer with a combination of light and photosensitizers.[24] Indeed, the photocytotoxicity of pheophorbide-type constituents on a panel of human tumor cell lines has been described.[25] Several nonmetal-chelated porphyrins were also reported to have anti-HIV activity and to bind to the V3 loop of the envelope glycoprotein gp120 of HIV-1.[26−28] The cytotoxic and antiviral effects of these compounds are most likely due to photodynamic destruction mediated by singlet oxygen[29] and superoxide radicals[30] that subsequently cause DNA, RNA, and protein damage via oxidative stress. In addition, direct electron transfer between DNA bases (especially guanine) and the pheophorbide singlet excited state may also contribute to DNA cleavage and subsequent cellular damage. These molecular events are not unique to pheophorbide compounds, but may be extended to porphyrins as well as to other photodynamic agents.

Subsequent studies revealed that while the free base analogues were photo-dependent cytotoxic agents, certain metal analogues of pheophorbide *a* and chlorin *e*6 exhibited potent but essentially photo-independent cytototoxic activity.[31] These metal-chelated compounds may be associated with novel mechanisms of action, including interaction with cellular DNA leading to the induction of double-stranded breaks.[31] The existence of photo-independent mechanism(s) of action for metal-chelated porphyrins was again underscored when the anti HIV-1 properties of heme were described.[32] In addition, heme and zinc protoporphyrin were observed to inhibit both HIV-1 and HIV-2 reverse transcriptase (RT) in a concentration-dependent manner.[33,34]

Under ordinary circumstances, special precautions are not employed to protect experimental cultures from light. In view of the fact that metalation of porphyrins is an enzyme-catalyzed reaction rather than a spontaneously occurring process, the role of metal ions as cofactors in the HIV-1 inhibitory effect of pheophorbide *a* in cell culture may be ruled out. The potent inhibitory effect presently observed with pheophorbide *a* (**13**) is most likely due to photoinactivation of cell-free virions and HIV entry receptors on the surface of HOG.R5 cells.[35] The minimal photoirradiation encountered under the ordinary circumstances of the experiment is unlikely to be sufficient to cause significant cellular toxicity at the concentrations of **13** tested.

Additional studies are needed to evaluate and further develop photo-independent pheophorbide *a* derivatives as antiviral and anticancer drug candidates. However, the clinical utility of pheophorbide-based photosensitizers is greatly restricted by its dependence on light penetration to the tissues involved. Nevertheless, photosensitization with these compounds may be a feasible approach to inactivation of transfusion-transmissible parasites and viruses in blood bank units.[36,37]

## Experimental Section

**General Experimental Procedures.** Optical rotations were measured on a Perkin-Elmer model 241 polarimeter. IR spectra were run on a Jasco FT/IR-410 spectrometer as a film on a KBr plate. 1D and 2D NMR spectra were recorded on a Bruker DRX-500 MHz spectrometer. Chemical shifts ($\delta$) were expressed in ppm with reference to the solvent signals. All NMR experiments were obtained by using standard pulse sequences supplied by the vendor. Column chromatography was carried out on Si gel (200−400 mesh, Natland International Corporation). Reversed-phase flash chromatography was accomplished with RP-18 Si gel (40−63 $\mu$m, EM Science), and reversed-phase HPLC was carried out on a Waters 600E Delivery System pump, equipped with a Waters 996 photodiode detector, and a GROM-Saphir 110 C18 column (120 Å, 12 $\mu$m, 300 × 40 mm). Thin-layer chromatography was performed on Whatman glass-backed plates coated with 0.25 mm layers of Si gel 60. HRTOFMS and MS/MS spectra were recorded on a Micromass QTOF-2 spectrometer. EIMS and HREIMS spectra were recorded on a Finnigan Mat 95 spectrometer.

**Plant Material.** The initial collection of leaf and twig samples of *Vatica cinerea* King was made at the Cuc Phuong National Park (CPNP), with voucher herbarium specimens represented by living collections in the park. A sample (2.7 kg) was collected for the isolation work. Voucher specimens (Soejarto et al. 11028) are on deposit at the herbaria of CPNP, Institute of Ecology and Biological Resources (National Center for Science and Technology, Hanoi), and the Field Museum of Natural History (Chicago, IL).

**Extraction and Isolation.** The dried, milled plant material (2.72 kg) was extracted with MeOH and concentrated. The resulting syrup (580 g) was subsequently defatted with petroleum ether and partitioned with $CHCl_3$. The $CHCl_3$-soluble fraction (26.5 g) was chromatographed over a Si gel column (500 g), which was developed by gradient elution with $CHCl_3$ and increasing concentrations of MeOH to afford 15 fractions. 3$\beta$-Glucositosterol (15.0 mg) was obtained from fraction F-11 by direct crystallization. Bioassay localized the anti-HIV activity in fractions F-3 (0.90 g), F-4 (0.52 g), F-5 (1.62 g), F-9 (2.59 g), and F-10 (1.85 g). Fractions F-3 to F-5 were pooled and subjected to flash column chromatography on Si gel (500 g, gradient elution with petroleum ether and $Me_2CO$) to yield the active fractions F-20 (690 mg) and F-21 (807 mg), which were combined and subjected to a C-18 reversed-phase (RP-18, 35 g) column. Elution of this column with MeOH and $H_2O$ yielded two purified active fractions, F-35 (585 mg) and F-36 (615 mg). Fraction F-36 was subjected to a series of preparative HPLC separations on a GROM-Saphir 110 C18 column (solvent system: either MeCN/$H_2O$, 9:1, or MeCN) to afford vaticinone (**1**, 0.9 mg), (23*E*)-27-*nor*-3$\beta$-hydroxycycloart-23-en-25-one (**2**, 3.5 mg), mangiferonic acid (**3**, 33.5 mg), dihydroschizandronic acid (**4**, 4.6 mg), (24*E*)-3-oxo-lanosta-8,24-dien-26-oic acid (**5**, 30.7 mg), dammara-20,25-dien-3$\beta$,24-diol (**6**, 4.0 mg), (23*E*)-dammara-20,23-dien-3$\beta$,25-diol (**7**, 4.2 mg), betulinic acid (**8**, 13.9 mg), betulin (**9**, 10.1 mg), betulonic acid (**10**, 1.6 mg), ursolic acid (**11**, 4.8 mg), and erythrodiol (**12**, 3.8 mg). Similar workup of fraction F-35 yielded (3*R*,6*R*,7*E*)-3-hydroxy-4,7-megastigmadien-9-one (0.9 mg), *N*-butylbenzenamine (4.7 mg), hydroxydihydrobovolide (2.4 mg), eudesm-4(15)-ene-1$\beta$,6$\alpha$-diol (4.3 mg), and 1-hydroxycyclocolorenone (**14**, 5.5 mg). Fractions F-9 and F-10, obtained from the initial Si gel column chromatographic development, were pooled and subjected to Si gel flash column chromatography (500 g, eluted with $CHCl_3$/$Et_2OAc$) to yield the active subfractions F-32 (440 mg), F-33 (122 mg), and F-34 (634 mg). These fractions were combined on the basis of their similar TLC profiles and subjected to flash chromatography on successive C-18 reversed-phase (RP-18: 35 and 107 g, respectively) columns (elution with $H_2O$/MeOH, MeOH, and $Me_2CO$) to afford the active fraction F-49 (184 mg). HPLC separation of F-49 on a GROM-Saphir 110 C18 column (solvent system: MeCN/$H_2O$, 9:1) led to isolation of the chlorophyll pheophorbide *a* (**13**, 10.4 mg).

**Vaticinone (1):** white powder, $[\alpha]_D^{20}$ +28.3° (*c* 0.06, $CHCl_3$); UV $\lambda_{max}^{MeOH}$ (log $\epsilon$) 198 (4.19), 221 (4.29) nm; IR (film) $\nu_{max}$ 3473, 2929, 1707, 1621, 1464, 1380, 1272, 1256, 1178, 1129, 1036, 986, 756 cm$^{-1}$; EIMS (70 eV) *m*/*z* 424 (54) $[M]^+$, 409 (35), 381 (24), 340 (23), 325 (12), 313 (30), 293 (26), 286 (52), 271 (18), 243 (13), 231 (11), 217 (16), 203 (79), 189 (30), 175 (39), 161 (35), 149 (100), 133 (53), 121 (75), 111 (65), 105 (56), 95 (73), 81 (50), 69 (37), 55 (56), 43 (64); HREIMS (70 eV) *m*/*z* 424.3350 $[M]^+$, calcd 424.3341 for $C_{29}H_{44}O_2$; $^1$H and $^{13}$C NMR data are listed in Tables 1 and 2.

**Dihydroschizandronic acid (4):** white powder, $[\alpha]_D^{20}$ +25.9° (*c* 1.51, $CHCl_3$); UV $\lambda_{max}^{MeOH}$ (log $\epsilon$) 201 (1.17) nm; $^1$H and $^{13}$C NMR data are listed in Tables 1 and 2.

**3-Oxo-24(*E*)-lanosta-8,24-dien-26-oic acid (5):** white powder, $[\alpha]_D^{20}$ +46.9° (*c* 1.51, MeOH); HR-TOF-MS *m*/*z* 453.3371 $[M - H]^+$, calcd 453.3369 for $C_{30}H_{45}O_3$; $^1$H and $^{13}$C NMR data are listed in Tables 1 and 2.

**Assay for the Inhibition of HIV Infectivity in HOG.R5 Cells.** Briefly, a reporter cell line for quantitating HIV-1 replication was developed using HOS (human osteosarcoma) cells rendered susceptible to HIV-1 infection by the transfection of genes for CD4 and CCR5, the coreceptor utilized by macrophage-tropic (R5) HIV-1 isolates. This microtiter assay is based on the transactivation of a stably integrated HIV-1 LTR-green fluorescent protein (GFP) transcription unit. Upon HIV-1 entry into these HOS target cells, Tat expression increases the HIV LTR-directed transcription of the GFP gene as demonstrated by the increased fluorescence of detergent lysates of infected cells relative to that of uninfected controls. Procedures adopted for the assay were as described previously.[38] The positive control compound used was 3TC (Lamivudine), which had an $IC_{50}$ value of approximately 1.2 $\mu$M in the HOG.R5 system utilizing the assay conditions described above. This nucleoside reverse transcriptase inhibitor and the virus stock of HIV-$1_{IIIB}$/H9 were obtained through the AIDS Research and Reference Reagent Program, Division of AIDS, NIAID, NIH.

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