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HIV INHIBITORY NATURAL PRODUCTS. 3.[1]

DITERPENES FROM *Homalanthus acuminatus* AND *Chrysobalanus icaco*

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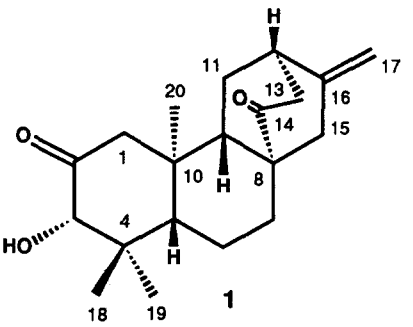
Summary. Extracts of the tropical rainforest trees *Homalanthus acuminatus* and *Chrysobalanus icaco* were active in the NCI AIDS-antiviral screen. Diterpenes 1-4 were found in *H. acuminatus*, while two (6,7) were found in *C. icaco*. Compounds 1 and 6 were active in the anti-HIV screen; 1, 3 and 4 were previously unknown.

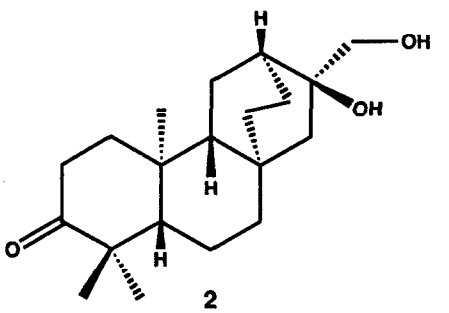
The National Cancer Institute has underway a major new intramural initiative to identify new anti-HIV and antitumor agents from natural sources.[2] As part of this effort, we recently reported the isolation and identification of prostratin[1], a potent HIV-inhibitory agent from extracts of the Samoan ethnobotanical tree *Homalanthus acuminatus*. During the final HPLC purification of prostratin, four additional diterpenes were obtained; one of these exhibited activity in the AIDS-antiviral screen[3]. Another tropical plant, *Chrysobalanus icaco*, provided two more diterpenes, one of which also proved inhibitory to HIV-1 infected cells *in vitro*.

For compound 1, EI-HRMS established the molecular formula, $C_{20}H_{28}O_3$, and infrared bands at 3491, 1713 and 1667 cm^{-1} were indicative of hydroxyl, carbonyl and olefinic functionalities, respectively. A ^1H NMR signal at δ 3.62 and a ^{13}C NMR methine signal at δ 82.6 revealed a secondary hydroxyl group. An exocyclic methylene (δ 147.7, 106.7) and two saturated ketones (δ 213.5, 209.6) accounted for three of seven degrees of unsaturation; therefore, 1 was tetracyclic. The ^1H NMR spectrum also contained three methyl singlets and two isolated pairs of methylene protons. Detailed ^1H-^1H COSY analysis, in conjunction with one-bond ^1H-^{13}C heteronuclear correlation experiments, established the presence of six proton (-CH-CH_2-CH-CH_2-) and five proton (-CH-CH_2-CH_2-) spin systems. These spectral features, along with recent reports of atisane diterpenes[4-8] in the taxonomically related genus *Euphorbia*, suggested that compound 1 was an atisane diterpene. Extensive long range (2-3 bond) heteronuclear correlation experiments were used to confirm the atisane skeleton and establish the functional group

substitution. Key correlations included those between the H-1 protons and C-2,3,5,10,20, between the H-17 protons and C-12,15 and between C-8 and the H-6,7,9,13,15 protons. Attempts to establish the complete relative stereochemistry through proton coupling constant analysis and nOe enhancement techniques left some ambiguities. Therefore, compound 1 was subjected to x-ray crystallographic analysis. A computer generated perspective drawing of the final x-ray model of compound 1 is given in Figure 1. The molecular geometry is unexceptional with the A and B rings having chair conformations and all six-membered rings of the bicyclo-[2.2.2]-octane, boat conformations. While the absolute stereochemistry of 1 was not rigorously defined, that proposed is consistent with other ent-atisane diterpenes from Euphorbia.[4-8]



1



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Compound 2, $C_{20}H_{32}O_3$ by HRMS, showed ^{13}C NMR resonances in $CDCl_3$ (see Experimental) that were virtually identical to those reported for ent-16S,17-dihydroxy-atisan-3-one[8]. ^{13}C and ^{1}H NMR assignments made in C_6D_6 using COSY and ^{1}H-^{13}C correlations were fully consistent with structure 2. A single-crystal x-ray structure determination of the 17-p-bromobenzoate of 2 had previously established the absolute configuration as that of an ent-atisane[8]. The co-occurrence of 1 and 2 in H. acuminatus further supports the assignment of the ent-atisane stereochemistry to 1.

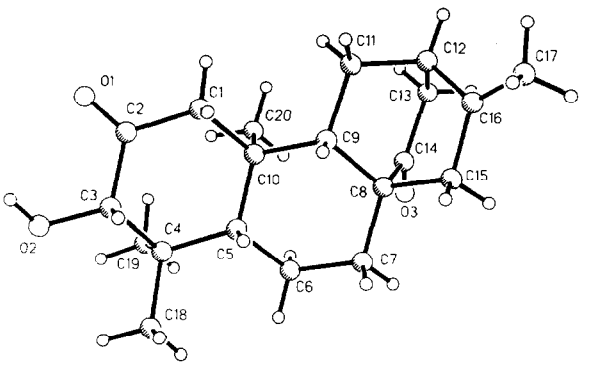


Figure 1. Computer Generated Perspective Drawing of the Final X-ray Model of 1.

Compound 3, $C_{20}H_{30}O_4$, had infrared absorbances at 3416 and 1713 cm^{-1} appropriate for hydroxyl and ketone groups. Nine of the ^{13}C NMR resonances closely corresponded to those observed for the A-ring carbons and methyl substituents of compound 1. Resonances for additional primary (δ 69.8) and tertiary (δ 79.1) hydroxyl groups suggested that the exocyclic

Table 1: 125 MHz ^{13}C NMR Data for Compounds **1-4** and **6-8**

Carbon #	1[a]	2[a]	3[a]	4[a]	6[b]	7[b]	8[b]
1	50.7 (2)	37.9 (2)	53.0 (2)	39.1 (2)	39.8 (2)	39.7 (2)	39.9 (2)
2	209.6 (0)[c]	34.0 (2)	210.4 (0)	33.9 (2)	18.7 (2)	18.8 (2)	18.8 (2)
3	82.6 (1)	217.4 (0)	82.7 (1)	215.6 (0)	37.5 (2)	37.7 (2)	37.9 (2)
4	45.3 (0)	47.4 (0)	45.1 (0)[d]	46.9 (0)	43.6 (0)	43.7 (0)	43.7 (0)
5	53.5 (1)	55.6 (1)	53.8 (1)	54.2 (1)	56.0 (1)	56.0 (1)	56.1 (1)
6	19.2 (2)	19.8 (2)	19.9 (2)	21.2 (2)	19.9 (2)	19.9 (2)	20.1 (2)
7	31.2 (2)	39.0 (2)	41.2 (2)	40.9 (2)	33.5 (2)	33.7 (2)	33.6 (2)
8	47.6 (0)	32.8 (0)	43.5 (0)	43.2 (0)	52.4 (0)	50.5 (0)	52.5 (0)
9	51.7 (1)	50.7 (1)	55.8 (1)	55.6 (1)	51.5 (1)	63.1 (1)	51.6 (1)
10	43.9 (0)	37.1 (0)	45.0 (0)[d]	38.4 (0)	40.2 (0)	38.8 (0)	40.1 (0)
11	27.4 (2)	23.1 (2)	18.7 (2)	19.2 (2)	18.3 (2)	66.2 (1)	18.3 (2)
12	38.5 (1)	32.5 (1)	26.4 (2)	26.9 (2)	32.1 (2)	41.1 (2)	32.2 (2)
13	44.3 (2)	23.4 (2)	40.9 (1)	41.0 (1)	38.0 (1)	36.8 (1)	38.1 (1)
14	213.5 (0)[c]	27.4 (2)	37.8 (2)	38.0 (2)	36.4 (2)	36.5 (2)	36.5 (2)
15	42.4 (2)	52.7 (2)	52.4 (2)	52.5 (2)	210.8 (0)	209.8 (0)	211.0 (0)
16	147.7 (0)	73.7 (0)	79.1 (0)	79.4 (0)	149.5 (0)	150.3 (0)	149.7 (0)
17	106.7 (2)	69.1 (2)	69.8 (2)	70.0 (2)	114.5 (2)	112.9 (2)	114.5 (2)
18	29.3 (3)	26.4 (3)	29.6 (3)	27.3 (3)	28.8 (3)	28.7 (3)	28.7 (3)
19	16.5 (3)	21.6 (3)	16.5 (3)	21.0 (3)	184.4 (0)	177.8 (0)	178.0 (0)
20	13.9 (3)	13.4 (3)	18.5 (3)	17.5 (3)	15.4 (3)	15.3 (3)	15.3 (3)
OMe						51.2 (3)	51.2 (3)

[a] C_6D_6, [b] $CDCl_3$, [c] Assignments made by correlation of A ring shifts with those of 3, [d] Assignments are interchangable

methylene present in 1 was oxidized to a vicinol diol in 3. Five proton (-CH-CH_2-CH_2-) and eight proton (-CH-CH_2-CH_2-CH-CH_2-) spin systems were distinguished by COSY, HMQC and HMBC experiments. These features could best be explained if compound 3 had a kaurane rather than atisane skeleton. Important two and three bond ^1H-^{13}C correlations supporting this assignment included those between the H-1 protons and C-2,3,5,10,20, between H-9 and C-1,8,10,12,14,20, between the H-14 protons and C-16 and between the H-15 protons and C-17. NOe enhancements between the diaxial H-3 and H-5 protons confirmed that the C-3 hydroxyl was equatorial. The relative stereochemistry at C-9 was defined by a strong nOe interaction between H-9 and H-1ß (δ 1.40) while an nOe enhancement between H-9 and H-15ß (δ 1.39) indicated that the C-15/C-16 bridge was linked to C-8 and C-13 in a ß-configuration. The orientation of the hydroxymethyl group at C-16 was revealed to be α by nOe enhancements between the H-17 protons and H-13, H-14ß (δ 0.72) and H-15α (δ 1.23). Assignment of the α and ß protons on C-14 and C-15 was aided by the W coupling (2.5 Hz) observed between H-14α and H-15ß in the ^1H NMR spectrum. All other nOe interactions and ^1H-^{13}C correlations were fully consistent with proposed structure 3 and a large negative optical rotation suggested the ent-kaurane configuration[9].

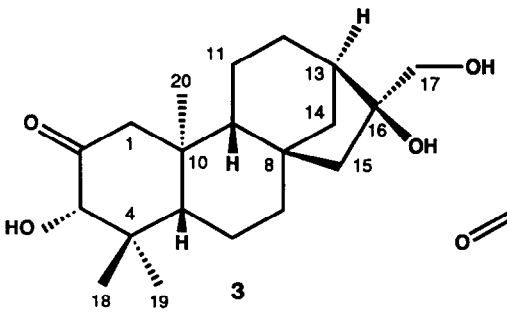
Compound 4 analyzed for $C_{20}H_{32}O_3$ by HRMS. ^{13}C and ^1H NMR spectra were obtained in $CDCl_3$ (see Experimental) for analysis and comparison with published data, while spectra recorded in

Table 2: 500 MHz ^{1}H NMR Data for Compounds 1-4 and 6-8

Carbon #	1[a]	2[a]	3[a]	4[a]	6[b]	7[b]	8[b]
1	1.34 (br d 11.5) 2.01 (d 12.3)	0.93[c] 1.40[c]	1.40 (br d 12.5) 2.49 (d 12.5)	0.99 (m) 1.62 (ddd 6.0, 6.5, 13.0)	0.77 (m) 1.78[c]	1.05[c] 1.90[c]	0.80 (dt 4.4, 12.8) 1.80[c]
2		2.18 (ddd 3.5, 6.0, 15.5) 2.29 (ddd 6.0,11.5, 15.5)		2.25 2H (m)	1.36 (m) 1.78[c]	1.46[c] 1.81 (tq 4.1, 13.6)	1.37[c] 1.80[c]
3	3.62 (br s)		3.61 (d 1.5)		0.97 (dt 4.5, 13.4) 2.10 (m)	1.03 (ddd 4.1, 12.4 13.6) 2.17 (m)	1.00 (dt 4.4, 13.4) 2.15 (m)
5	0.88 (dd 2.5, 12.3)	1.00 (dd 3.5, 13.5)	0.93 (dd 2.0, 12.0)	1.08 (br d 12.5)	1.13 (m)	1.20 (m)	1.13 (dd 2.4, 12.0)
6	1.34 (m) 1.54 (dq 4.0, 13.2)	1.14 2H (m)	1.05 (dq 3.5, 13.0) 1.33(m)	1.18 2H (m)	1.75[c] 1.84 (m)	1.69 (dq 2.9, 12.2) 1.95 (m)	1.73 (m) 1.91 (dq 2.4, 13.0)
7	0.50 (dt 4.9, 13.2) 2.31 (ddd 2.7, 4.0, 13.2)	0.91[c] 1.13[c]	1.20 2H (m)	1.19 2H (m)	1.29 (m) 1.78[c]	1.40 (m) 1.90[c]	1.34 (dt 2.9, 13.0) 1.83[c]
9	1.07 (dd 6.5, 10.8)	1.07 (m)	0.90 (br d 8.0)	0.89 (br d 8.5)	1.17[c]	1.38[c]	1.20 (br d 8.54)
11	0.90 (ddd 2.6, 6.4, 13.7) 1.22 (ddt 3.1, 10.8, 13.7)	0.92[c] 1.91 (ddt 3.0, 11.4, 14.0)	1.18 (m) 1.98 (m)	1.35 (m) 2.13 (m)	1.41 (m) 1.60[c]	4.02 (d 4.8)	1.41 2H (m)
12	2.16 (quint. 3.0)	1.64 (m)	1.25 (m) 1.73 (m)	1.37 (m) 1.87 (m)	1.60[c] 1.78[c]	1.90[c] 2.07 (ddd 2.9, 4.8, 14.2)	1.64 (m) 1.80[c]
13	1.84 (dd 2.7, 18.9) 2.00 (dt 2.8, 18.9)	1.22 (m) 1.40 (m)	1.87 (m)	2.02 (m)	2.98 (m)	3.03 (m)	3.02(m)
14		0.50 (m) 1.56 (ddt 3.5, 12.0, 14.0)	0.72[c] 1.57 (dd 2.5, 12.0)	0.81 (m) 1.70m (dd 2.5, 12.0)	1.32[c] 2.33 (d 11.8)	1.44[c] 2.32 (d 12.2)	1.39[c] 2.35 (d 12.0)
15	1.79 (dt 2.1, 17.6) 1.97 (dt 2.4, 17.6)	0.97 2H (m)	1.23 (d 14.5) 1.39 (dd 2.5, 14.5)	1.29 (d 14.0) 1.44 (dd 2.5, 14.0)			
17	4.54 (dd 2.1, 3.8) 4.75 (dd 2.4, 3.8)	3.14 (d 10.5) 3.26 (d 10.5)	3.09 (d 11.0) 3.12 (d 10.5)	3.22 (d 10.5) 3.28 (d 10.5)	5.19 (t 1.0) 5.87 (t 1.0)	5.24 (t 1.5) 5.84 (t 1.5)	5.22 (d 1.0) 5.91 (d 1.0)
18	1.08 3H (s)	1.10 3H (s)	1.11 3H (s)	1.02 3H (s)	1.20 3H (s)	1.18 3H (s)	1.17 3H (s)
19	0.60 3H (s)	0.92 3H (s)	0.68 3H (s)	0.97 3H (s)			
20	0.46 3H (s)	0.77 3H (s)	0.74 3H (s)	0.80 3H (s)	0.96 3H (s)	0.81 3H (s)	0.89 3H (s)
OMe						3.63 3H (s)	3.63 3H (s)

[a] C_6D_6, [b] $CDCl_3$, [c] Overlapped resonance

C_6D_6 (Tables 1 and 2) provided increased signal dispersion. Compound **4** showed ^{13}C NMR resonances ($CDCl_3$) which closely matched the reported values for **5**, ent-16R,17-dihydroxykauran-3-one[10]. However, the significant differences observed for C-16 (δ 79.6) and C-17 (δ 69.8) in **4** relative to the corresponding C-16 (δ 81.6) and C-17 (δ 66.0) positions in **5** suggested that the C-17 hydroxymethylene of **4** was α. This assignment was supported by shifts in the ^1H NMR ($CDCl_3$) resonances of the H-17 protons in **4** (δ 3.39, 3.47) relative to those in **5** (δ 3.68, 3.79). NOe enhancements between the H-17 protons and H-15α (δ 1.29) provided further evidence for the S configuration at C-16. COSY and ^1H-^{13}C correlation experiments clearly defined the kaurane skeleton. Key long range correlations included those between C-16 and H-14α (δ 1.70), between C-17 and H-15α, between C-4 and the H-18 and H-19 methyls, and between the H-20 methyl and C-1,9,10. All nOe interactions, including those between H-9 and H-5, H-11B (δ 2.13) and H-15B (δ 1.44), supported the relative stereochemistry proposed for **4**. The large negative optical rotation of **4** suggested that it had the ent-kaurane configuration.



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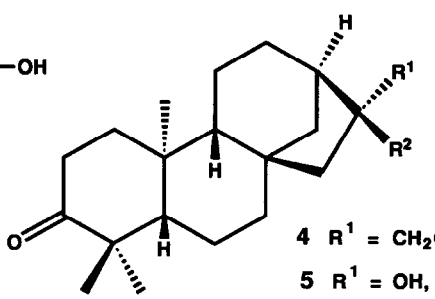


4 $R^1 = CH_2OH$, $R^2 = OH$

5 $R^1 = OH$, $R^2 = CH_2OH$

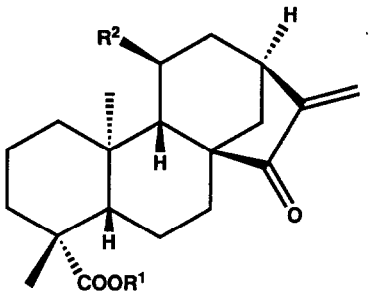
The organic extract of Chrysobalanus icaco also showed an inhibitory effect on in vitro HIV viral infection. Bioassay guided fractionation led to the major diterpene component **6** and the accompanying trace constituent **7**.

The structure of **6** was established as ent-15-oxo-kaur-16-en-19-oic acid by comparison of its spectral data to published values[12,13]. Further confirmation was obtained by conversion to the methyl ester **8**, whose spectral data also correlated well with literature values[14,15].

The minor metabolite **7** analyzed for $C_{21}H_{30}O_4$ by HRMS and had a 3H singlet at δ3.62 in the ^1H NMR, indicative of a methyl ester. A ^1H doublet at δ4.0 (J=2.1 Hz) and a methine carbon signal at 66.2 ppm revealed the presence of a secondary alcohol in **7**. Extensive NMR studies, including nOe and heteronuclear correlation experiments, clearly demonstrated that **7** was the 11-hydroxyl methyl ester analog of **6**. Observation of nOe enhancements of the H-20 angular methyl and both H-12 protons following irradiation of H-11 clearly established the B orientation of the 11-hydroxyl group. Additional nOe interactions between the H-20 methyl and the H-14α proton (δ 2.32) defined the five membered ring junction as B.

This is the first report of compound **7** as a natural product. It had previously been reported as a methylation product of the corresponding hydroxy acid[16]. The spectral characteristics of **7** were only briefly described and the ^1H NMR data did not match our data. While the NMR solvent for the literature values of **7** was not specified, $CDCl_3$ or pyridine-d_5

were utilized for all other compounds in that report[16]. Our ^1H NMR data for **7** in either of these solvents were clearly different from the published values. No ^{13}C NMR data were previously provided for **7**. While the reason for this discrepancy is not clear, the data we report are fully consistent with **7**.



6 R^1 = H, R^2 = H
7 R^1 = Me, R^2 = OH
8 R^1 = Me, R^2 = H

Compounds **1-4** and **6-8** were tested for in vitro anti-HIV activity in the NCI's XTT tetrazolium assay[3]. At a concentration of 6 μg/ml, compound **1** provided a maximum of 50% protection to HIV infected cells, and was non-cytotoxic to uninfected control cells. However, at 12 μg/ml cytotoxic effects became appparent in both control and HIV-infected cultures. A similar activity profile, but greater potency, was seen with compound **6** (non-cytotoxic and approximately 50% protective at 0.5 μg/ml, but cytotoxic at 2 μg/ml). While the diterpene acid **6** showed these modest antiviral effects, that activity was completely absent in the methyl ester derivative **8**. Compounds **2-4** and **7** did not inhibit HIV infection, but were cytotoxic at concentrations ranging from 2-50 μg/ml. While compounds **1** and **6** do not show sufficient potency or therapeutic indices in our primary assays to support their candidacy for drug development, they nonetheless provide new leads for more detailed biological characterization and prioritization for synthetic and/or semisynthetic modifications aimed at enhancing their therapeutic potential.

EXPERIMENTAL

General: Large-scale separations were performed with a Waters C_{18} Prepak^R 500 cartridge and final purification was effected on a Rainin Dynamax^R silica column (2.1 x 25 cm). NMR spectra were recorded on a Varian VXR 500 spectrometer using C_6D_6 or $CDCl_3$ as solvent and internal standard; the attached proton numbers for the ^{13}C-NMR signals were determined from DEPT experiments. Infrared spectra were measured on a Perkin-Elmer 1600 FTIR spectrometer. Optical rotations were measured on a Perkin-Elmer 241 polarimeter. Mass spectra were recorded on a VG Micromass ZAB 2F mass spectrometer.

Isolation of the *Homalanthus acuminatus* diterpenoids. The collection and extraction of stem wood of H. acuminatus and solvent partitioning of the resulting extract have been described[1]. A large recollection (9.4 kg) was extracted in the same general manner to give 99.3g crude extract. The $CHCl_3$ soluble partition fraction was separated by gradient elution vacuum liquid chromatography on silica gel using increasingly polar mixtures of hexane/EtOAc/MeOH. The anti-HIV activity was concentrated in fractions eluted with 60% EtOAc/hexane through 100% EtOAc. These fractions were combined (7.8 g) and passed through a column of Sephadex LH-20 using 1:1 CH_2Cl_2/MeOH. The active fractions (5.6 g total) were separated by preparative HPLC using an H_2O/MeOH gradient. Final purification was achieved by silica HPLC (hexane-EtOAc) to provide **1** (67 mg), **2** (82 mg), **3** (26 mg), and **4** (13 mg).

Isolation of the *Chrysobalanus icaco* diterpenoids. The roots of the tree C. icaco were collected in the Central African Republic and extracted as described above. A portion of the

crude organic extract (2.11 g) was fractionated by flash chromatography on C-18 packing[11]. The material eluted with 90:10 MeOH/H_2O and 100% MeOH was combined (906 mg) and chromatographed on a diol flash column. The CH_2Cl_2 and 9:1 CH_2Cl_2/EtOAc fractions were combined and crystallized from hexane/Et_2O to give **6** (210 mg). HPLC of the mother liquors on C_{18} provided **7** (13 mg).

Anti-HIV assay. DMSO solutions of compounds **1-4** and **6-8** were tested in the XTT based in vitro anti-HIV assay, experimental details of which have been reported previously[3].

ent-3S-hydroxy-atis-16(17)-en-1,14-dione (1). Prisms from hexane/EtOAc, mp 155°;$[\alpha]_D$ = -15.7° (c = 5.8,$CHCl_3$); EI-HRMS: m/z 316.1997, $C_{20}H_{28}O_3$ requires 316.2038; IR (film) 3491, 3084, 2970, 1713, 1667, 1438 cm^{-1}.

ent-16S,17-dihydroxyatisan-3-one (2). $[\alpha]_D$ = -43° (c = 0.5, $CHCl_3$); EI-HRMS: m/z 320.2314, $C_{20}H_{32}O_3$ requires 320.2351; IR (film) 3400, 2934, 2879, 1703, 1450 cm^{-1}; ^{13}C NMR (125 MHz, $CDCl_3$) δ 13.4 (3), 19.6 (2), 21.6 (3), 22.9 (2), 23.2 (2), 26.1 (3), 27.1 (2), 32.1 (1), 32.8 (0), 34.0 (2), 37.2 (0), 38.0 (2), 38.7 (2), 47.6 (0), 50.8 (1), 52.4 (2), 55.6 (1), 69.0 (2), 74.0 (0), 217.5 (0).

ent-3S,16S,17-trihydroxy-kauran-2-one (3). mp 140-142°, $[\alpha]_D$ = -58.5° (c = 2.2, $CHCl_3$); EI-HRMS:m/z 336.2292, $C_{20}H_{32}O_4$ requires 336.2301; IR (film) 3416, 2937, 1713, 1448, 1391 cm^{-1}.

ent-16S,17-dihydroxykauran-3-one (4). $[\alpha]_D$ = -73.1° (c = 1.8, $CHCl_3$); EI-HRMS: m/z 320.2324, $C_{20}H_{32}O_3$ requires 320.2351; IR (film) 3400, 2936, 1692 cm^{-1}. ^1H NMR ($CDCl_3$), δ 1.01 3H (s), 1.06 3H (s), 1.08 3H (s), 3.39 (d, 10.5), 3.47 (d, 10.5); ^{13}C NMR ($CDCl_3$) δ 17.6 (3), 19.2 (2), 20.9 (3), 21.2 (2), 26.5 (2), 27.3 (3), 34.0 (2), 37.8 (2), 38.5 (0), 39.2 (2), 40.6 (1), 40.8 (2), 43.2 (0), 47.1 (0), 42.2 (2), 54.3 (1), 55.6 (1), 69.8 (2), 79.6 (0), 218.2 (0).

ent-15-oxo-kaur-16-en-19-oic acid (6). mp 174-6°; $[\alpha]_D$ = -145.8° (c = 8.0, $CHCl_3$) [Lit[12,13] mp 168-173°; $[\alpha]_D$ = -160°]; IR (film) 3250-2600, 1725, 1693, 1645, 1447 cm^{-1}: EI-HRMS: m/z 316.2032, $C_{20}H_{28}O_3$ requires 316.2038.

Methylation of 6. A stirred Et_2O solution of **6** (20 mg) was treated with excess CH_2N_2 at 0° for 1 hr. HPLC purification of the reaction product on silica [hexane/EtOAc, 19:1] afforded **8**, 13 mg, EI-HRMS: m/z 330.2193, $C_{21}H_{30}O_3$ requires 330.2195; IR (film) 2932, 1725, 1645, 1446 cm^{-1}.

ent-11S-hydroxy-15-oxokaur-16-en-19-oic acid methyl ester (7). $[\alpha]_D$ = -158.8° (c = 0.8, $CHCl_3$); EI-HRMS: m/z 346.2116, $C_{21}H_{30}O_3$ requires 346.2144; IR (film) 3490, 2933, 1725, 1646, 1446 cm^{-1}; ^{13}C NMR (125 MHz, pyridine-d_5) δ 15.6 (3), 19.3 (2), 20.6 (2), 28.6 (3), 34.7 (2), 37.2 (2), 37.7 (1), 38.0 (2), 38.9 (0), 39.9 (2), 41.5 (2), 43.8 (0), 50.8 (0), 51.2 (3), 56.2 (1), 63.0 (1), 110.9 (2), 151.8 (0), 177.6 (0), 208.8 (0).

Single crystal x-ray diffraction analysis of ent-3S-hydroxy-atis-16(17)-en-2,14-dione (1). Single crystals of **1** suitable for an x-ray diffraction investigation were obtained from a hexane-ethyl acetate solution by slow evaporation. These rectangular plates formed in the monoclinic space group $P2_1$ with a = 6.2936(3), b= 19.1776(6), c= 14.1718 (4) Å and β = 99.418(4)° and two independent molecules of composition $C_{20}H_{28}O_3$ in the asymmetric unit. Diffraction data with $2\theta \leq 45°$ were collected using MoKα radiation and θ:2θ scans at -45°C. After correction for Lorentz, polarization and background effects, 1761 (61%) of the 2292 independent reflections were judged observed ($|F_o| \geq 4\sigma(|F_o|)$) and used in subsequent calculations. The structure was solved by direct methods and refined with full-matrix least-squares refinement with anisotropic heavy atoms and fixed isotropic riding hydrogens to a conventional crystallographic residual of 5.08%.[17] Both molecules had the same conformation and only one is illustrated in Figure 1.

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