Antiinflammatory *C*-Glucosyl Chromone from *Aloe barbadensis*

John A. Hutter,[†] Mohammad Salman,[‡] William B. Stavinoha,[†] Neera Satsangi,[†] Robert F. Williams,[‡] Robert T. Streeper,[§] and Susan T. Weintraub*,[§]

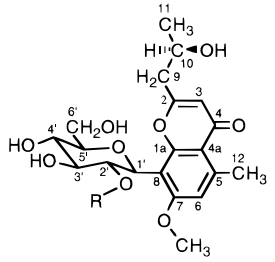
Department of Pharmacology, Research Imaging Center, and Department of Biochemistry, The University of Texas Health Science Center at San Antonio, 7703 Floyd Curl Drive, San Antonio, Texas 78284-7760

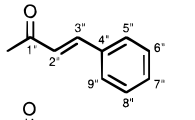
Received December 27, 1995[⊗]

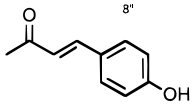
A new antiinflammatory agent identified as 8-[*C*-*β*-D-[2-*O*-(*E*)-cinnamoyl]glucopyranosyl]-2-[(*R*)-2-hydroxypropyl]-7-methoxy-5-methylchromone (**1**) has been isolated from *Aloe barbadensis* Miller. At a dose of 200 *μ*g/mouse ear, **1** exhibited topical antiinflammatory activity equivlaent to 200 *μ*g/ear of hydrocortisone. There was no reduction in thymus weight caused by treatment with **1** for any of the doses tested, while 200 *μ*g/ear of hydrocortisone resulted in a 50% decrease in thymus weight.

Aloe spp., commonly known as aloe vera, have a long history as a home remedy for burns and for treatment of a variety of conditions in traditional medicine. There is also widespread use of aloe vera in cosmetics and skin lotions. In view of the large body of anecdotal evidence attesting to the efficacy of substances obtained from the leaves of *Aloe* spp., we have initiated a study of antiinflammatory components of *Aloe barbadensis* Mill. (Liliaceae). This report describes the structural elucidation and evaluation of antiinflammatory activity of a *C*-glucosylchromone isolated by reversed-phase HPLC.

The new *C*-glucosylchromone isolated from *A. barbadensis* eluted as a single peak with a retention time of 27 min by analytical reversed-phase HPLC using a mobile phase of 50% aqueous MeOH. The off-white solid that was recovered was characterized by UV, MS, and NMR analyses, resulting in the identification of this substance as 8-[*C*-*β*-D-[2-*O*-(*E*)-cinnamoyl]glucopyranosyl]-2-[(*R*)-2-hydroxypropyl]-7-methoxy-5-methylchromone (**1**).



1 R = 

2 R = 

3 R = H

The UV spectrum of **1** in MeOH exhibited four principal absorption maxima (log ϵ), 220 nm (4.74), 246 nm (4.60), 254 nm (4.66), and 284 nm (4.74), in general agreement with the values reported by Speranza and co-workers for aloeresin D (**2**).[1]

Analysis of **1** by EIMS indicated a molecular weight of 540 Da. The fragmentation of **1** by EIMS was identical to **2** for the chromone portion of the molecule.[1] In contrast, the fragment ions attributable to the acyl substituent on the sugar were 16 mass units lower than the *p*-coumaroyl fragments in **2**, consistent with the presence of a cinnamoyl moiety in **1**. Acetylation of **1** was accomplished by treatment at room temperature overnight with a mixture of acetic anhydride and pyridine (1:1, v/v). The product was analyzed by electrospray ionization MS (ESIMS) by infusion in 70% aqueous MeOH/0.5 mM NaOAc. The ESI mass spectrum was characterized by an intense peak at *m*/*z* 731, consistent with the addition of four acetyl groups (4 × 42 Da = 168 Da) plus a sodium ion (23 Da) to yield $[M + Na]^+$. The ESIMS results confirmed the molecular weight of **1** as 540 Da.

Alkaline hydrolysis of **1** (using the conditions described by Speranza and co-workers[1] for the hydrolysis of **2**) afforded the corresponding alcohol, **3**, which was found to be identical to the chromone released by alkaline hydrolysis of **2**[1] on the basis of UV, ^{1}H and ^{13}C NMR, and MS analysis. Furthermore, exposure of **1** to anhydrous methanol/anhydrous HCl resulted in the formation of methyl cinnamate as indicated by EIMS analysis and comparison of the HPLC retention time with that of authentic methyl (*E*)-cinnamate. In concert, these results support the identification of **1** as the cinnamoyl analog of **2**.

Examination of **1** by ^{1}H and ^{13}C NMR was also undertaken. The results of these analyses are in agreement with the proposed structure of **1** and with the published ^{1}H and ^{13}C NMR spectra of **2** in methanol-d_4[2] and DMSO-d_6.[1] The data support a *β*-*C*-glucosyl moiety at position 8 of the chromone nucleus and substitution of an (*E*)-cinnamoyl group at carbon-2 of the sugar residue. In particular, in the aromatic region of the ^{1}H NMR spectrum of **1**, the absence of the AA′BB′ pattern characteristic of a *p*,*p*-disubstituted aryl group and the presence of a complex set of closely-spaced peaks integrating for five protons support the identification of a cinnamoyl substituent in **1** as compared to the coumaroyl moiety in **2**. Moreover, the large *J* value

* Author to whom correspondence should be addressed. Tel.: (210) 567-4043. Fax: (210) 567-5524. E-mail: weintraub@uthscsa.edu.
† Department of Pharmacology.
‡ Research Imaging Center.
§ Department of Biochemistry.
⊗ Abstract published in *Advance ACS Abstracts,* April 15, 1996.

S0163-3864(96)00151-6 CCC: $12.00

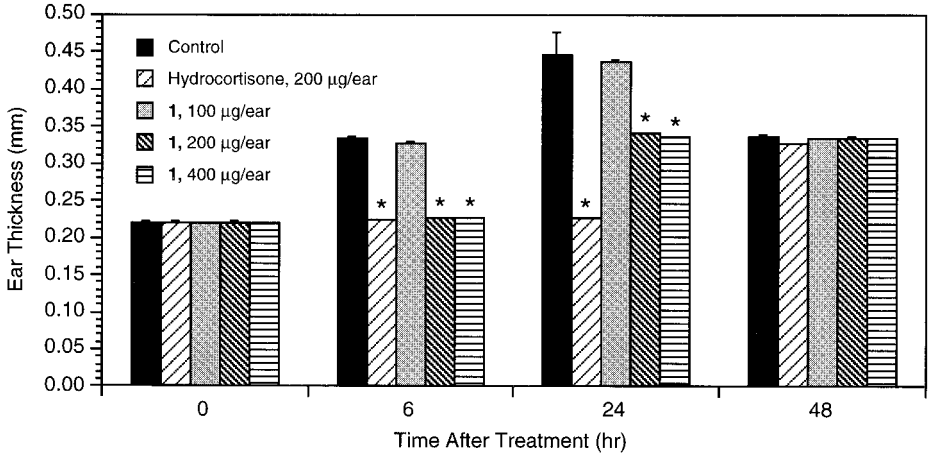


Figure 1. Topical antiinflammatory activity of **1**. Each bar represents the mean ± s.d. for three animals: *, $p < 0.0001$, statistically different from control as assessed by a one-way analysis of variance and comparison of means through the use of StatView (Abacus Concepts, Berkeley, CA).

(16.2 Hz) observed for the vinylic protons is consistent with a *trans* configuration for the 2″ double bond.

In view of the structural similarities between **1** and **2** as determined by spectral analysis (NMR and MS), an *R* configuration has been assigned to carbon 10 of **1**. However, lack of sufficient quantities of purified **1** has prevented the determination of absolute configuration at this time. The chirality at C-10 will be fully investigated in the future when more material becomes available.

Assessment of the topical antiinflammatory activity of **1** was then made through use of an *in vivo* mouse assay[3] in which the ability of **1** to reduce croton oil-induced ear inflammation was compared with hydrocortisone. As can be seen in Figure 1, a dose of 200 μg/ear of **1** afforded antiinflammatory activity that was comparable to 200 μg of hydrocortisone at 6 h after treatment; there was diminished residual activity of **1** at 24 h. Of particular importance is the fact that there was no reduction in thymus weight caused by treatment with **1** for any of the doses tested while 200 μg/ear of hydrocortisone resulted in a 50% decrease in thymus weight (mean ± standard deviation for three animals per group: control, 24.5 ± 3.2 mg; **1** (400 μg/ear), 22.1 ± 2.0 mg; hydrocortisone (200 μg/ear), 12.5 ± 0.4 mg). These results support the efficacy of aloe vera as a topical antiinflammatory agent and indicate that the cinnamoyl-*C*-glucosylchromone described in this paper is a significant contributor to the biological activity of *A. barbadensis*.

Experimental Section

General Experimental Procedures. Electron impact ionization mass spectra were acquired on a Finnigan MAT 4615 quadrupole mass spectrometer at an electron energy of 70 eV and an ion source temperature of 160 °C. Sample introduction was by means of either gas chromatograph or direct insertion probe which was heated ballistically until evaporation was complete. Electrospray ionization mass spectra were obtained on a Finnigan MAT SSQ700 quadrupole mass spectrometer fitted with an Analytica of Branford electrospray interface. An electrospray energy of −3.5 kV was employed. Samples were infused into the mass spectrometer at a flow rate of 1 μL/min in a solution of 0.5 mM sodium acetate in 70% aqueous methanol.

Proton and ^{13}C NMR analyses were conducted on a General Electric Model QE 300 spectrometer. Samples were dissolved in either methanol-d_4 or DMSO-d_6, as indicated. The chemical shift values are expressed as δ (ppm) relative to tetramethylsilane (for ^1H) or solvent (for ^{13}C) which were used as internal standards.

Analytical HPLC was performed on a Hewlett-Packard Model 1090 liquid chromatograph under control of a Hewlett-Packard Chem Station. HPLC conditions were as follows: column, Ranin Dynamax, C18, 4.6 × 250 mm, 60 Å, 8 μm particle size; mobile phase, 50% aqueous methanol at a flow rate of 1 mL/min; detector wavelength, 280 nm.

Plant Material. *A. barbadensis* was collected from the fields of Aloecorp (Harlingen, TX); a voucher specimen is deposited at the Plant Resources Center herbarium of the University of Texas at Austin (Austin, TX).

Isolation of 1. *A. barbadensis* Miller leaves (10 kg) were cut into segments of approximately 0.5 in. diameter prior to grinding in a blender. The resulting slurry was filtered and the filtrate treated with activated carbon. After isolation by vacuum filtration, the carbon-containing residue was washed with deionized water and then extracted with EtOH. The solvent was removed *in vacuo*, and the residue was mixed with water and extracted with methylene chloride. The residue that remained after evaporation of the organic phase was extracted with MeOH. The resulting components in the MeOH extract were fractionated by preparative reversed-phase HPLC. The yield of **1** was 131 mg.

1: off-white solid; mp 138−139 °C (previous softening, 125 °C); UV (MeOH) λ_{max} (log ϵ) 220 (4.74), 246 (4.60), 254 (4.66), 284 (4.74) nm; ^1H NMR (CD_3OD, 300 MHz) δ 1.31 (3 H, d, J = 6.0 Hz, H-11), 2.72−2.91 (2 H, m, H-9), 2.74 (3 H, s, H-12), 3.41−3.55 (1 H, m, H-5′), 3.62 (1 H, dd, J = 9.3, 9.3 Hz, H-4′), 3.70−3.81 (2 H, m, H-3′, H-6′a), 3.89 (3 H, s, 7-OCH_3), 3.97 (1 H, d, J = 15 Hz, H-6′b), 4.34−4.45 (1 H, m, H-10), 5.20 (1 H, d, J = 10.2 Hz, H-1′), 5.74 (1 H, dd, J = 9.3, 9.3 Hz, H-2′), 6.13 (1 H, s, H-3), 6.27 (1 H, d, J = 16.2 Hz, H-2″), 6.82 (1 H, s, H-6), 7.30−7.48 (3 H, m, H-5″, H-7″, H-9″), 7.40 (1 H, d, J = 16.2 Hz, H-3″), 7.50−7.60 (2 H, m, H-6″, H-8″); ^1H NMR (DMSO-d_6, 300 MHz) δ 1.17 (3 H, d, J = 6.0 Hz, H-11), 2.56−2.81 (2 H, m, H-9), 2.67 (3 H, s, H-12),

3.35−3.50 (1 H, m, H-5′), 3.50−3.65 (1 H, m, H-4′), 3.70−3.83 (2 H, m, H-3′, H-6′a), 3.85 (3 H, s, 7-OCH_3), 3.87 (1 H, d, J = 13.8 Hz, H-6′b), 4.10−4.25 (1 H, m, H-10), 4.99 (1 H, d, J = 10.2 Hz, H-1′), 5.60 (1 H, dd, J = 9.9, 9.7 Hz, H-2′), 6.06 (1 H, s, H-3), 6.37 (1 H, d, J = 16.2 Hz, H-2″), 6.85 (1 H, s, H-6), 7.34−7.42 (3 H, m, H-5″, H-7″, H-9″), 7.40 (1 H, d, J = 16.2 Hz, H-3″), 7.60−7.68 (2 H, m, H-6″, H-8″); ^{13}C NMR (DMSO-d_6, 75 MHz) δ 22.8 (C-12), 23.3 (C-11), 43.1 (C-9), 56.5 (7-OMe), 61.5 (C-6′), 64.4 (C-10), 70.4 (C-4′), 70.6 (C-1′), 72.6 (C-2′), 75.7 (C-3′), 81.8 (C-5′), 110.6 (C-8), 111.0 (C-6), 111.3 (C-3), 115.8 (C-4a), 117.8 (C-2″), 128.2 (C-6″, C-8″), 128.9 (C-5″, C-9″), 130.4 (C-7″), 133.9 (C-4″), 141.7 (C-5), 144.1 (C-3″), 157.4 (C-1a), 159.5 (C-7), 165.0 (C-1″), 165.0 (C-2), 178.6 (C-4); EIMS (70 eV) m/z $[M]^+$ 540 (12), $[M - H_2O]^+$ ($[M - 18]^+$) 522 (5), $[M - CH_3CHO]^+$ ($[M - 44]^+$, derived through α-cleavage of the 2-hydroxypropyl side chain) 496 (4), [M − cinnamoyl]$^+$ ($[M - 130]^+$, involves H transfer to the charge retaining fragment) 410 (16), $[M - (130 + 18)]^+$ 392 (10), [M − (cinnamate + 18)]$^+$ ($[M - (146 + 18)]^+$, involves H transfer) 376 (4), $[M - (130 + 44)]^+$ 366 (8), 343 (18), $[M - (133 + 130)]^+$ (the 133-mass unit fragment corresponds to C2−C6 of the C-glucoside) 277 (50), $[M - (133 + 130 + 18)]^+$ 259 (100), $[M - (133 + 130 + 44)]^+$ 233 (92), $[M - (133 + 130 + 84)]^+$ (the 84-mass unit fragment is generated by retro-Diels−Alder fragment that includes the hydroxypropyl side chain) 193 (73), [cinnamoyl]$^+$ 131 (40), $[C_6H_5CHCH]^+$ 103 (33).

Animals. Male BalbC mice weighing 20−25 g were obtained from Harlan Sprague−Dawley (Madison, WI). Randomly chosen mice were housed five to a cage and maintained on a light cycle of 7 am to 9 pm. All mice were allowed free access to Harlan Lab Chow LM485 and water.

Measurement of Antiinflammatory Activity. Topical activity was assessed by the method of Carlson.[3] In order to induce ear swelling, 10 μL of croton oil solution (25 mg of croton oil dissolved in 1 mL of pyridine/water/diethyl ether (4:1:5, v/v/v)) was applied by means of an Eppendorf pipette to the inner side of the left ear of each mouse. Each test material was dissolved in the same vehicle and applied to the same location on the left ear, 30 min after croton oil treatment. Animals treated with hydrocortisone were evaluated in parallel as a model for potent antiinflammatory activity. Ear swelling in a control group in which animals were treated with croton oil alone was also assessed. Ear thickness was measured by means of an Oditest caliper. Measurement was made before application of croton oil and at 6, 24, and 48 h after treatment with the test solutions.

Acknowledgment. The authors are grateful to Aloecorp for financial support and to Todd Waller of Aloecorp for supplying the starting material for this investigation. J.A.H. thanks Dr. B. E. Haskell, University of Texas, Austin, TX, for help on this project.

References and Notes

(1) Speranza, G.; Dada, G.; Lunazzi, L.; Gramatica, P.; Manitto, P. *Phytochemistry* **1986**, *25*, 2219−2222.
(2) Manitto, P.; Monti, D.; Speranza, G. *Gazz. Chim. Ital.* **1990**, *120*, 641−646.
(3) Carlson, R. P.; O'Neil-Davis, L.; Chang, J.; Lewis, J. A. *Agents Actions* **1985**, *17*, 197−204.

NP9601519